Switch of histamine receptor expression from H2 to H1 during differentiation of monocytes into macrophages

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Abstract It is known that histamine suppresses gene expression and synthesis of tumor necrosis factor alpha (TNF-α) induced by lipopolysaccharide (LPS) in human peripheral blood mononuclear monocytes (HPM) or alveolar macrophages via histamine H2 receptors. We investigated the effect of histamine and differentiation in macrophages on the expression and secretion of TNF-α, TNF-α-converting enzyme (TACE), and histamine H1 and H2 receptors by use of a leukemia cell line, U937, and HPM. Differentiation of U937 and HPM cells with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) enhanced the H1 receptor expression and rather suppressed the H2 receptor, resulting in up-regulation of the histamine-induced expression and secretion of TNF-α, modulated via TACE. Therefore, histamine failed to inhibit up-regulated expression of TNF-α induced by LPS in macrophages. The switch from H2 to H1 receptors during differentiation in the monocyte/macrophage lineage could participate in the pathogenic processes of atherosclerosis and inflammatory reactions in the arterial wall.

Key words: Histamine H1 and H2 receptor; Tumor necrosis factor alpha; Tumor necrosis factor alpha converting enzyme; U937; Peripheral blood monocyte/macrophage lineage; Lipopolysaccharide; Atherosclerosis

1. Introduction

Histamine has long been considered to be an important mediator of certain types of inflammation including allergic reactions [1–3], a neurotransmitter [4], a modulator of gastrointestinal function [5], and vascular permeability [6], and to exert direct effects on cardiovascular hemodynamics [7]. Recently, a growing number of reports indicate effects of histamine on cell adhesion and growth [8–11]. These effects of histamine are mediated by three specific kinds of surface receptors, i.e. H1, H2 and H3 subtypes, which were identified firstly on the basis of their pharmacological properties and later in terms of their DNA sequences [12–14]. Histamine H1 receptors are known to be coupled with the phosphoinositol hydrolysis pathway [15]; H2 receptors with adenylate cyclase [13]; and H3, probably with G proteins [14]. In a previous paper [15], we demonstrated for the first time a large number of histamine H1 receptors in arterial smooth muscle and endothelial cells and showed that the expression of these receptors could be stimulated by platelet-derived growth factor (PDGF), suggesting that histamine is involved not only in pathogenesis and progression of atherosclerosis but also in physiological mechanisms of arteries via H1 receptors. However, little is known about the role of histamine in the function of macrophages, which is an other important cell type that plays an essential role in atherosclerosis and secretes TNF-α. It was reported that histamine inhibited TNF-α expression, which contributes essentially to processes of septic shock and inflammation, in human peripheral monocytes and human alveolar macrophages via H2 receptors [16,17]. Since macrophages in atherosclerotic foci are derived from peripheral monocytes [18], it is necessary for a better understanding of the mechanism of initiation and progression of atherosclerosis to examine the relationship between macrophage maturation and TNF-α, TNF-α converting enzyme (TACE), and H1, 2, and 3 receptor gene expression. In the present study, we investigated and discussed the relationship between the expression of histamine receptors and the maturation from monocytes to macrophages and discussed the role of histamine receptors in the pathogenesis in the arterial wall by using U937 cells, which differentiate along the macrophage lineage in response to a variety of stimuli such as phorbol esters [19], and HPM.

2. Materials and methods

2.1. Cell cultures

U937 histiocytic lymphoma line from the American Type Culture Collection (Rockville, MD, USA) was maintained in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS) purchased from INC Biochemical INC (Irvine, CA, USA), 100 U/ml penicillin and 100 ng/ml streptomycin. ISS10 cells (SMC), which are human intimal smooth muscle cells immortalized by SV 40 [15], were also used in experiments as a control. The cells were grown in 90-mm^2 culture dishes at 37°C in a humidified atmosphere of 5% CO_2/95% air in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical, Tokyo, Japan). Human peripheral blood mononuclear cells were isolated from the blood of three healthy donors (medical students in our university) by using Lymphoprep™ (Nycomed Pharmas AS, Oslo, Norway) as a separation medium. The blood was diluted by addition of an equal volume of 0.9% NaCl and

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layered over Lymphprep™ in 50-ml tubes and then centrifuged at 800×*g* for 20 min at room temperature; and then the sample/medium interface was harvested. After two washes with 0.9% NaCl to remove the platelets, the cells were cultured in RPMI 1640 (Gibco, Grand Island, NY, USA) containing 10% FCS. After cultivation for 24 h, floating cells were carefully removed and used for experiments.

2.2. Assay of TNF-α

For differentiation of U937 cells, the cells (5×10^5/well) were pre-incubated in six well plates with 10 ng/ml of TPA for 24 h, and the medium was exchanged for 1 ml of TPA-free fresh medium containing various concentrations of histamine (Wako Pure Chemical Ind., Osaka, Japan), 10 ng/ml of TPA (Sigma, St. Louis, MO, USA), and/or 1 μg/ml of lipopolysaccharide (LPS) purchased from Difco Lab. (Detroit, MI, USA). After isolation from peripheral blood, cells of the monocyte/macrophage lineage underwent spontaneous differentiation into macrophages in the TPA-Free medium as described previously [20]. After cultivation for 24–96 h, triplicate cultures were terminated by removal of the supernatants, which was followed by centrifugation (900×*g*, 10 min, 4°C); and then TNF-α in the supernatant was measured. Immunoreactive TNF-α was assayed by a sandwich ELISA (Cytokin ELAISA kit; Toyobo, Tokyo, Japan). Samples were incubated with anti-hTNF-α antibody bound to the assay plate. After the plate had been washed with the buffer, biotinylated antibody specific for hTNF-α was added; and then the excess second antibody was removed, and streptavidin-peroxidase was added. After a third incubation and washing to remove the unbound enzyme, a substrate solution was added to visualize the bound enzyme. The intensity of the colored product was directly proportional to the concentration of hTNF-α present in the original specimen.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

The cells were cultured under the same conditions mentioned above for the assay of TNF-α. First we extracted the total RNA from untreated or treated cultured cells, purified it with Traizol reagent (Gibco BRL, Grand Island, NY, USA), and employed reverse transcription in the presence of human histamine H1, H2 and H3 receptor, TNF-α, or TACE gene-specific primers: H1, 5′-TAAGCTGAGGCCAGAGAACC and 3′-TACTGTCTTGAATGCGAGCG; H2, 5′-GCACAGCCTCCATTCTTAACC and 3′-CCTCATTGATGGCATCATCC; H3, 5′-TCCTCTGCCTTCAACATCG and 3′-ATCATCAGCAGCGTGTATGG; TNF-α, 5′-GGCAGTCAGATCATCTTCTCG and 3′-ATGTTCGTCCTCCTCACAGG; and TACE, 5′-TGAGCAGCATGGATTCTGC and 3′-TGTCAACACGATTCTGACGC. Total RNA (50 μg) was exposed to SuperScript II RNaseH$^-$ reverse transcriptase (1000 U) (Gibco BRL) in the presence of oligo (dT) primer (500 ng), dNTP (0.9 mM each), and first strand buffer in a total volume of 50 μl. The reaction was allowed to continue for 1 h at 37°C [21]. A 1-μl aliquot of each reaction mixture was then subjected to PCR using each specific primer. Denaturation, annealing and extension temperatures and times were 94°C for 1 min, 60°C for 30 s, and 72°C for 1 min, respectively, for 35 cycles. On completion of PCR, the amplified DNA fragments were then visualized with a UV transilluminator. DNA fragments of RT-PCR products for H1, H2, and H3 receptor, and TNF-α, and TACE genes (492, 498, 779, 491, and 309 bp, respectively) were subcloned in a pGEM-T Easy vector (Promega, Madison, WI, USA), and the purified DNAs were analyzed with an ALF DNA sequencer (Pharmacia Biotech, Uppsala, Sweden) to confirm their specific sequences [22].

2.4. Northern blot analysis

RT-PCR products mentioned above were directly extracted and purified using a QIAquik PCR Purification kit (QIAGEN, Valencia, CA, USA) for use in Northern blotting as specific probes. These DNA fragments were labeled with [α-^{35}P]dCTP by use of a BcaBEST Labeling kit (TaKaRa, Ohtu, Japan) and separated from unincorporated ^{32}P-labeled nucleotides on a NICK Column (Pharmacia Biotech). Total RNA (10 μg) from each untreated or treated culture was loaded onto a 1.4% agarose/formaldehyde denaturing gel, blotted onto Hybond-N nylon filters (Amersham, Buckingamshire, UK), and prehybridized for 3 h at 42°C with 50% formamide, 5×standard citrate (SSC; 1× = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 2×Denhardt's solution (1× = 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), 0.1% SDS, and 0.1 mg/ml of denatured herring sperm DNA. Then the filters were hybridized for 48 h with each specific probe.

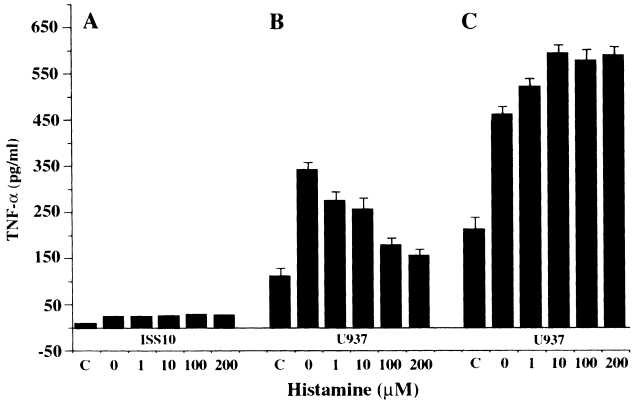

Fig. 1. Effect of histamine on production of TGF-α by U937 cells treated with TPA and LPS. A: After a 24 h-cultivation of U937 or SMC with 10 ng/ml TPA, 1 μg/ml of LPS, and various concentrations of histamine were added; and the secretion of TNF-α was analyzed 24 h later. In cultured SMC as a control, TNF-α secretion was slightly and synergistically increased in a dose-dependent manner. B: Histamine exerted, however, a dose-dependent inhibitory effect on the secretion of TGF-α in U937 cells induced by LPS at 24 h under the same culture conditions as in A. C: However, when U937 cells were incubated continuously for 3 days after the treatment with TPA for 24 h, histamine failed to suppress the up-regulation of TNF-α by LPS in the cells and rather stimulated it synergistically, even under TPA-free culture conditions.

3. Results and discussion

3.1. Induction of TNF-α in relation to differentiation in the monocyte/macrophage lineage

Vannier et al. reported from their study on human peripheral peripheral blood mononuclear monocytes that histamine suppressed the gene expression and synthesis of TNF-α induced by LPS [16]. Our results from the assay of TNF-α (Fig. 1) show that SMC, used as a control, could secrete a small amount of TNF-α, whose secretion was slightly stimu-

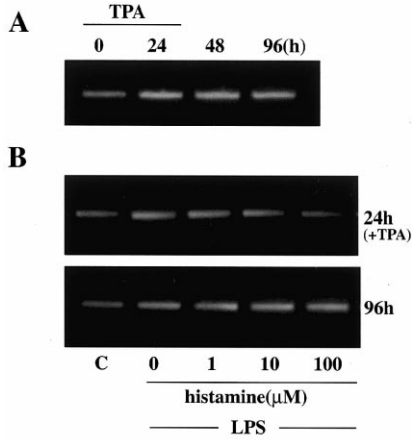

Fig. 2. Effect of histamine on expression of the TACE gene in U937 cells. A: RT-PCR examination showed that the cultivation of U937 cells with TPA alone induced up-regulation of the TACE gene expression within 24 h and that the up-regulation was continuously detected for 3 days, even under TPA-free culture conditions. B: After the treatment of U937 cells with TPA for 24 h, the stimulation of TACE induced by LPS was inhibited by histamine within 24 h, whereas when U937 cells were incubated for an additional 3 days under TPA-free culture conditions after treatment with TPA for 24 h, the expression of the TACE gene was continuously increased and no inhibitory effect of histamine was detected.

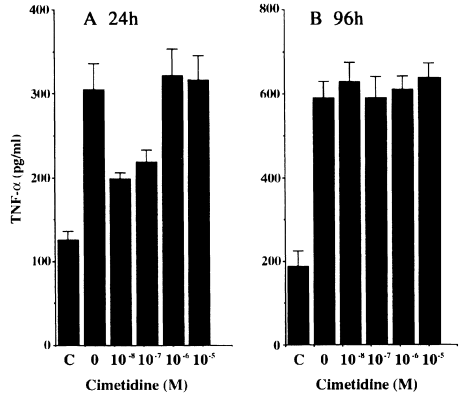

Fig. 3. Effect of histamine H2 blocker on TGF-α secretion in U937 cells treated with TPA, LPS, and histamine. A: When cimetidine was added to U937 cells at the same time as 1 μg/ml of LPS and 100 μM histamine after the preincubation with TPA for 24 h and these cells were incubated for 24 h, 10^{-6}–10^{-5} M cimetidine inhibited the inhibitory effect of histamine on TNF-α secretion induced by LPS in the cells. B: However, when the cells were incubated continuously for 3 days under TPA-free culture conditions after treatment with TPA for 24 h, no inhibitory effect of cimetidine was detected.

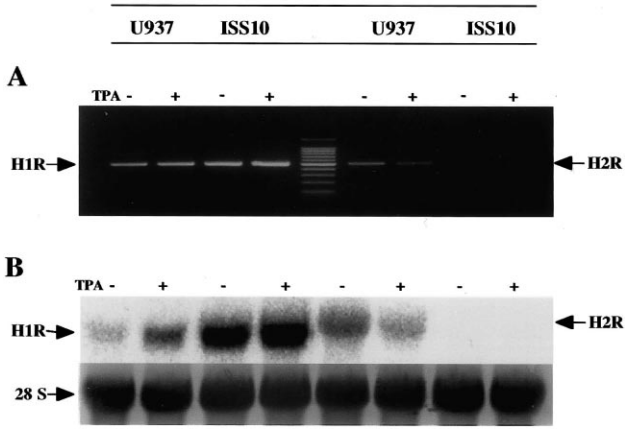

Fig. 4. Effect of TPA on expression of histamine receptor expression in U937 cells and SMC. A: RT-PCR reveals that treatment with 10 ng/ml TPA alone for 24 h enhanced the expression of histamine H1 receptors in both U937 and SMC cells, whereas TPA suppressed histamine H2 receptors expressed in U937 cells. H2 receptors were not expressed in SMC. B: Northern blot examination reveals results similar to those of RT-PCR analysis.

lated by histamine, and that after incubation with 10 ng/ml of TPA for 24 h, U937 cells secreted a certain amount of TNF-α, which became pronounced by 1 μg/ml of LPS, whereas the up-regulation of TNF-α secreted induced by LPS was suppressed by histamine. These data on U937 cells are consistent with those reported previously [16,17]. In addition, TNF-α secretion induced in U937 cells was increased twofold 96 h after incubation with TPA for 24 h, and was markedly pronounced by addition of LPS, whereas histamine fails to suppress and rather stimulated TNF-α secretion synergistically. Since U937 cells treated with TPA differentiate into macrophages, these results suggest that the effect of histamine on macrophages differs from that on monocytes.

3.2. A role for TACE in up-regulation of TNF-α

The TNF-α-converting enzyme (TACE) can process a membrane-bound TNF-α precursor of $M_r = 26$ kDa to generate the secreted mature TNF-α of $M_r = 17$ kDa [23]. As shown in Fig. 2, RT-PCR of TACE gave similar results as those of TNF-α: differentiation of U937 cells in the presence of 10 ng/ml of TPA for 24 h enhanced TACE gene expression, which increased as the cultivation period was increased, even in the absence of TPA. The up-regulation was even more pronounced by the addition of 1 μg/ml LPS; and in the cells incubated with TPA for 24 h, histamine suppressed the up-regulation, whereas it rather stimulated the TACE expression in cultivated cells for 3 days after a 24-h stimulation with TPA, indicating that up-regulation of secreted TNF-α by these agents involves TACE action and that the effect of histamine on the expression of TACE differs between macrophages and monocytes. We reported previously that SMC could produce matrix metalloproteinases (MMPs), the production of which was stimulated with TPA, PDGF, histamine, epidermal growth factor, and calcium ionophore [24,25] and that histamine enhanced phosphoinositide hydrolysis, increased cytoplasmic Ca^{2+} level, and stimulated the transcription of the c-fos protooncogene via H1 receptors [10]. TACE is a member of the MMP family, which contain AP-1 sites in their promoter region of most of the members studied [26]. AP-1 sites, however, are not found in the 5′-flanking region of the mouse TACE gene but in a GC-box, which seems to be the major site [23]. Since Masuda et al. reported that a *cis*-acting region, i.e. the GM-kB/GC-box, is required for maximal induction by stimulation of TACE by phorbol ester and calcium ionophore in T cells [27], we suggest that histamine may stimulate TACE expression via Ca^{2+} as a second messenger in macrophages via H1 receptors.

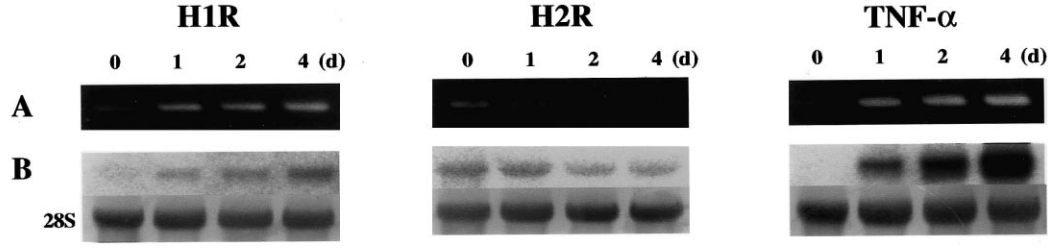

Fig. 5. Time course of H1 and H2 receptor expression and TNF-α expression in U937 cells. The cells were incubated with 10 ng/ml of TPA for 24 h and then continuously cultured without TPA for 3 days. RT-PCR and Northern blot analyses show an increase in H1 receptor but a decrease in H2 receptor expression in U937 cells treated with 10 ng/ml of TPA, which changes occurred in a time-dependent manner. The amount of TNF-α expression was also markedly stimulated time dependently.

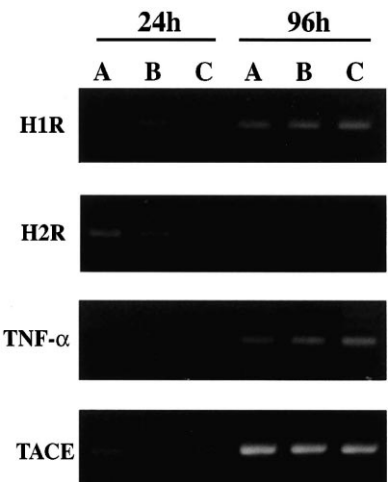

Fig. 6. Expression of the H1 and H2 receptors, TNF-α, and TACE genes in HPM. RT-PCR reveals the expression of the H2 receptor gene in monocytes 24 h after isolation from the peripheral blood of three healthy donors and that it decreased during a 96-h cultivation, whereas at 96 h after isolation from peripheral blood, expression of the histamine H1 receptor, TNF-α, and TACE genes was pronounced.

3.3. Switch of histamine receptor type in relation to differentiation in the monocyte/macrophage lineage

Vanniei et al. demonstrated that the suppression of TNF-α gene expression in human monocytes by histamine was modulated by H2 receptors [16]. Previously we reported that human arterial SMC expressed strongly histamine H1 but not H2 receptors [10], indicating that the production of TGF-α secreted from SMC in response to histamine was stimulated via H1 receptors. In addition, we presently found that cimetidine, a histamine H2 blocker, inhibited the suppression of TNF-α activity by histamine (Fig. 3) in U937 cells after incubation with TPA for 24 h. Therefore, we investigated the relationship between the expression of histamine receptors and the differentiation of the monocyte/macrophage lineage. The results of RT-PCR and Northern blot analyses are shown in Fig. 4, which reveal an increase in H1- and a decrease in H2 receptor expression in U937 cells after incubation of the cells with TPA for 24 h but only up-regulation of H1 receptor in SMC was noted. The switch of histamine receptors occurred within 1 or 2 days after the treatment with TPA (Fig. 5). These results indicate that the switch of histamine receptor expression from H2 to H1 receptors took place in relation to the differentiation of the monocytes into macrophages. In our experimental conditions, no RT-PCR product for H3 receptor was detected in these cells.

3.4. Involvement of switch of histamine receptors in human atherosclerosis

After isolation from human peripheral blood and as the cultivation time was increased, cultured HPM underwent more definitive differentiation into macrophages without TPA. This switching of histamine receptors in relation to differentiation was also confirmed by our study on HPM (Fig. 6). In our previous paper, we suggested that histamine H1 receptors expressed in arterial intimal SMC could participate in the progression of atherosclerosis [15]. Macrophages play an essential role in the progression of atherosclerosis; and many macrophages, which phagocytose large amounts of lipid, are detected in atherosclerotic foci [18]. Our results suggest that atherosclerosis may be able to accelerate the processes of inflammatory reaction and septic shock, in which TNF-α is a key factor, and that atherosclerosis itself can be accelerated by pronounced inflammatory reactions in the arterial wall via histamine H1 receptors of macrophages.

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