Increase in histamine content and enhancement of high affinity IgE receptor Fc ϵ RI expression in the human leukemia KU812 cells upon treatment with hydrocortisone

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Abstract

Hydrocortisone was investigated for its ability to differentiate human leukemia KU812 cells into mature hematopoietic cells including basophils. Hydrocortisone treatment increased the amount of intracellular histamine by up-regulation of L-histidine decarboxylase (HDC) mRNA and enhanced cell surface expression of the high affinity IgE receptor Fc ϵ RI. Histamine is catalyzed from L-histidine by HDC, which in blood cell types is only expressed in basophils and mast cells. Cells, on which the Fc ϵ RI expression was enhanced by hydrocortisone, were shown to release histamine when stimulated with anti-IgE antibody after sensitization with myeloma IgE, implying that the induced Fc ϵ RI molecules were able to transduce a signal for degranulation. These results suggest that hydrocortisone promotes differentiation of KU812 cells into functionally mature basophilic cells.

Introduction

Glucocorticoids are effective in reducing inflammation and in suppressing immune responses through multiple mechanisms, including the modulation of cytokine production, phagocytosis, antigen presentation, migration, and cytotoxicity in many different cell types. For this reason, glucocorticoids are widely used therapeutically for the intervention of allergic diseases. As effector cells in the IgE-dependent immune response, basophils and mast cells appear to play an important role by producing and releasing mediators such as histamine, leukotriene and cytokines during an allergic reaction. It has been reported that glucocorticoids have inhibitory effects on histamine release and leukotriene formation in human basophils after being stimulated with IgE and specific antigen (Schleimer et al., 1981; Zor et al., 1987; Crocker et al., 1997). Several *in vivo* studies revealed that glucocorticoids block late phase allergic responses, which is characterized by a migration and a recruitment of basophils, as well as lymphocytes and eosinophils,

into sites of inflammation (Bascom et al., 1988; Charlesworth et al., 1991). In contrast, glucocorticoids have little or no effect on IgE-stimulated mediator release from mast cells (Schleimer et al., 1983; Cohan et al., 1983). Furthermore, it has been reported that the addition of dexamethasone, a synthetic glucocorticoid, to mouse bone marrow-derived mast cells (mBMMC) induces an increase in cellular histamine content and an appearance of granulated cells morphologically similar to mature mast cells (Pitton et al., 1988). Another experiment showed that when mBMMC cells were treated with dexamethasone, increased mRNA levels for mouse mast cell carboxypeptidase A (MC-CPA) and serglycin, a proteoglycan found in mast cell granules, were observed (Eklund et al., 1997). These suggest that glucocorticoids can, at least partially, induce differentiation in mast cell. However, only limited information is available concerning the effect of glucocorticoids on basophil differentiation.

In this study, we evaluated the efficacy of hydrocortisone, an endogenous glucocorticoid, on the differentiation of the human pre-basophilic cell line

KU812. This cell line was originally established from a patient with chronic myelogenous leukemia (Kishi, 1985) and has exhibited the potential to differentiate into mature hematopoietic cells including basophils (Navarro et al., 1990; Valent et al., 1990; Nilsson et al., 1994; Hara et al., 1998). We demonstrate here that hydrocortisone treatment resulted in the increase in the amount of intracellular histamine, elevated level of HDC mRNA, and the enhancement of cell surface expression of Fc ϵ RI in KU812 cells.

Materials and methods

Reagents

Hydrocortisone, histamine dihydrochloride and *o*-phthalaldehyde were purchased from Wako (Osaka, Japan). Mouse anti-human Fc ϵ RI monoclonal antibody CRA1 (Ra et al., 1993; Yanagihara et al., 1994; Toru et al., 1996) was obtained from Kyokuto seiyaku (Tokyo, Japan). Human myeloma IgE antibody was bought from Cosmo Bio Co. (Tokyo, Japan). FITC-conjugated goat anti-human IgE antibody and unconjugated mouse anti-human IgE antibody were purchased from Biosource (Camarillo, CA) and Cappel (Durham, NC), respectively. FITC-conjugated mouse anti-human CD23 antibody was obtained from Ancell (Bayport, MN). Mouse IgG2b antibody and FITC-conjugated mouse IgG1 antibody for negative control were bought from Dako (A/S, Denmark). The calcium ionophore A23187 was purchased from Sigma Chemical Co. (St Louis, MO). Tyrode buffer, containing 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 11.9 mM NaHCO₃, 0.4 mM NaH₂PO₄, and 5.6 mM glucose (pH 7.2), was prepared for use in the histamine release assay.

Cells and cell culture

KU812 cells were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan) and were maintained in RPMI-1640 (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (Intergen, Purchase, NY), 100 U/ml penicillin G, 100 mg/ml streptomycin and 10 mM HEPES buffer. KU812 cells were cultured at 37 °C in a humidified atmosphere with 5% CO₂.

Treatment of KU812 cells with hydrocortisone

Hydrocortisone was dissolved in ethanol, handled un-

der subdued light conditions and was diluted 1000-fold into medium so that the final ethanol concentration was 0.1%. At this concentration, ethanol showed almost no effect on cell growth and viability compared with medium alone. KU812 cells were cultured with or without various concentrations of hydrocortisone or ethanol (vehicle) alone. Cells were inoculated at 2.5×10^5 cells/ml and maintained for 21 days, medium changes were done every 5 days. Cell numbers were estimated using an electronic particle counter (Sysmex F-520, Toa iyoudenshi, Kobe, Japan). Viability was determined by trypan blue dye exclusion.

Wright-Giemsa and toluidine blue staining

Cells were centrifuged and resuspended in fresh RPMI-1640 medium at a concentration of about 5.0×10^7 cells/ml. This cell suspension was placed on glass slides, allowed to air dry, and then stained with Wright reagent (Wako, Osaka, Japan) at a 2/3 dilution in methanol for 10 min followed by staining with Giemsa reagent (Wako) at a 1/20 dilution in sodium phosphate buffer (pH 6.8) for 20 min (Wright-Giemsa staining). Alternatively, the slide preparations were subjected to staining with 1% toluidine blue (ICN, Costa Mesa, CA) in methanol (pH 2.5) for 1 h (toluidine blue staining).

Histamine measurement

Histamine content was measured by fluorometric assay (Shore et al., 1959). To determine the amounts of total cellular histamine, KU812 cells (1.0×10^6 cells) were suspended and sonicated in Tyrode buffer. Then, the cell lysates were centrifuged at $10\,000 \times g$ for 10 min and the supernatants were harvested. After mixing 2 ml of the supernatant with 0.75 g of NaCl and 0.5 ml of 1 N NaOH, 5 ml of the 3:2 (v/v) mixture of *n*-butanol and chloroform was added, and mixed for 5 min. The solution was centrifuged for 5 min at $300 \times g$ and 4 ml of the upper organic solvent layer was recovered. The recovered layer was mixed with 2 ml of *n*-heptane and 1.5 ml of 0.1 N HCl. After centrifugation at $300 \times g$ for 5 min, 1 ml of the lower HCl layer was recovered, mixed with 0.15 ml of 1 N NaOH and the subsequent product was added with 0.1 ml of 0.2% *o*-phthalaldehyde dissolved in methanol for 5 min at room temperature to yield a fluorescent product. The reaction was terminated by adding 0.14 ml of 0.5 N H₂SO₄ and fluorescence intensity was measured using a spectrofluorophotometer

(RF1500, Shimadzu, Kyoto, Japan) with the excitation set at 360 nm and the emission set at 450 nm. Histamine content was quantitated by comparison to a standard curve generated by coupling histamine dihydrochloride to *o*-phthalaldehyde.

Analysis of HDC mRNA expression by reverse transcription-polymerase chain reaction (RT-PCR) and Southern blotting

Total cellular RNA was isolated using TRIZOL reagent (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's instructions. For cDNA synthesis, 10 μ g of total RNA was denatured at 70 °C for 10 min, quickly chilled on ice for 10 min, and reverse transcribed in the reaction volume of 20 μ l containing 0.5 μ g of oligo dT20 (1 μ g/ml), 20 U of Moloney mouse leukemia virus (MMLV)-reverse transcriptase (Amersham, U.K.), dNTP and 0.2 μ l RNase inhibitor (Takara, Shiga, Japan) in reaction buffer, incubated at 37 °C for 60 min. Reactions were terminated by heating at 95 °C for 5 min and the sample was then stored at -20 °C. The resultant cDNA samples were subjected to 11 cycles of PCR amplification in the presence of specific sense and antisense primers for HDC (1 μ M each), 200 μ M dNTP, 0.05 U/ μ l Taq DNA polymerase (Fermentas, Lithuania) and PCR buffer (1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.8, 0.08% Nonidet P40). Human glyceraldehyde-3-phosphoryl dehydrogenase (G3PDH) cDNA was amplified as an internal control, and distilled water substituting the template cDNA was used as a negative control. PCR was performed for 11 cycles, each cycle including denaturation at 94 °C for 30 s, annealing at 50 °C (HDC) or 60 °C (G3PDH) for 30 s, and polymerizations at 72 °C for 60 s. Sequences for PCR primers and sizes for expected products are as follows: for HDC: sense 5'-AATCTTCAAGCACATGTC-3', and antisense 5'-CTGGATAGTGGCCGGGATGA-3' (207 bp); for G3PDH: sense 5'-CATCACCATCTTCCAGGAGC-3', and antisense 5'-GGATGATGTTCTGGAGAGCC-3' (404 bp). For Southern blotting, the amplified PCR products were subjected to electrophoresis on a 1.5% agarose gel, transferred onto a Hybond-N⁺ membrane (Amersham). Blots were prehybridized, and then hybridized with probes specific for HDC (internal probe: 5'-TGCCAAGAGGCACCTTGGCCTGGT-3') or G3PDH (random labeling probe). After probing, the membranes were washed as recommended by the

manufacturer. The hybridized probe was detected using Gene Images detection kit (Amersham).

Flow cytometric analysis of FcεRI expression

Expression of cell surface FcεRI was assessed by indirect immunofluorescence and flow cytometry. In brief, cells were incubated with CRA1 or human myeloma IgE for 60 min at 4 °C. Then the cells were washed twice in phosphate-buffered saline (PBS; pH 7.5) and exposed to FITC-conjugated F(ab')₂ goat anti-mouse IgG antibody or FITC-conjugated goat anti-human IgE antibody for 60 min at 4 °C. Cells were washed twice in PBS and subjected to flow cytometry (FACSCalibur; Becton Dickinson, Sunnyvale, CA). As a negative control, mouse subclass-matched polyclonal IgG2b antibody or purified human serum IgG, replacing CRA1 or human myeloma IgE respectively, was used. To detect CD23, direct immunofluorescence was performed using mouse anti-CD23 antibody and subclass-matched FITC-conjugated IgG1 antibody as a negative control. The percentage of positive cells in the staining pattern for CRA1 was calculated with the arbitrary 1% cutoff channel position determined by the negative control.

Histamine release

KU812 cells (1.0×10^6 cells) were washed and resuspended in 2 ml of Tyrode buffer. Five hundred μ l of 25 μ M (final, 5 μ M) A23187 or 10 μ g/ml (final, 2 μ g/ml) goat anti-human IgE antibody after sensitization with 25 μ g/ml (final, 5 μ g/ml) of human myeloma IgE at 37 °C for 4 h was added to the cell suspension. The mixture was incubated at 37 °C for 20 min and the reaction was terminated by cooling at 4 °C for 15 min. Then the cell suspension was centrifuged at $300 \times g$ for 10 min, and the amount of histamine in the supernatant was measured by fluorometric assay as described above.

Statistical analysis

Results were expressed as mean $\pm$ standard error (SE) ($n = 5$). If error bars are shown, they represent the SE. For evaluation of significant differences between the groups, statistical analysis was performed using the Student's *t*-test in each experiment.

Figure 1. Effect of hydrocortisone on the morphology of KU812 cells. (A) Morphology of immature cells (A, C) and basophilic cells showing cytoplasmic granules (B, D) in culture. Basophilic granules were stained dark-reddish purple by Wright-Giemsa staining (A, B) or pink-purple by toluidine blue staining (C, D). (E) The percentages of granulated cells in the KU812 cells cultured in medium only (None) or in the presence of 0.1% ethanol (EtOH) or 1 μ M hydrocortisone (HC). At certain time points (i.e., days 0, 7, 14 and 21), the cells were subjected to Wright-Giemsa (open bar) and toluidine blue (closed bar) staining. Results are expressed as the mean $\pm$ SE (n = 5).

Results

Effect of hydrocortisone on the morphology and growth of KU812 cells

The most distinct morphological feature for mature basophils is the presence of cytoplasmic granules, in which heparin proteoglycan is stored. We therefore

assessed the effect of hydrocortisone on the granulation of KU812 cells by Wright-Giemsa staining and toluidine blue staining, which are commonly used techniques for studying the morphology of blood cells and mature basophils, respectively. It is known that the cytoplasmic granules of mature basophils can be stained dark reddish-purple with Wright dye metachromatically. Under hydrocortisone-free culture con-

Figure 2. Growth of KU812 cells cultivated in medium only (open circle) or in the presence of 0.1% ethanol (closed triangle) or 1 μ M hydrocortisone (closed circle). At certain time points (i.e., days 0, 5, 10, 15 and 20), the cell numbers were measured. Results are expressed as the mean $\pm$ SE ($n = 5$).

ditions, there were very low percentages of granulated cells (<0.5% of total cells). A typical mature basophilic cell showing cytoplasmic granules is shown in Figure 1B. During a 21 day culture period, treatment with 1 μ M hydrocortisone had no appreciative effect on the population of granulated cells (Figure 1E). Alternatively, toluidine blue staining also revealed that there was no significant change in the percentage of the cells exhibiting basophilic granules stained pink-purple in the presence of hydrocortisone (Figures 1C–E).

In order to maintain the experimental culture, medium was changed and cell density was reduced to an inoculation density of 2.5×10^5 cells/ml every 5 days, and at these time points cell numbers were estimated. When cultivated with hydrocortisone at a concentration of 1 μ M, growth of KU812 cells was slightly enhanced by approximately 1.2-fold (Figure 2). At a lower concentration than 1 μ M, hydrocortisone had almost no effect on the growth of KU812 cells (data not shown). No inhibitory effect on the cell growth was observed in the presence of 0.1% ethanol. The percentage of viable cells was more than 98% under all culture conditions throughout the experiments.

Effect of hydrocortisone on the amount of intracellular histamine and mRNA level of HDC in KU812 cells

To examine whether hydrocortisone effects the production of histamine in KU812 cells, cells were cultivated for up to 21 days with or without various concentrations of hydrocortisone. Before the experi-

Figure 3. Dose- (A) and time- (B) dependent increase in the amount of intracellular histamine in KU812 cells by hydrocortisone. (A) Cells were maintained in the absence or presence of various concentrations of hydrocortisone for 21 days. (B) Cells were cultivated in medium only (open circle) or in the presence of 0.1% ethanol (closed triangle) or 1 μ M hydrocortisone (closed circle). At certain time points (i.e., days 0, 3, 7, 14 and 21), the cells were lysed by sonication and the amount of intracellular histamine was measured. Results are expressed as the mean $\pm$ SE ($n = 5$).

ment, KU812 cells were shown to maintain a constant level of histamine (mean $\pm$ SE = 6.9 ± 0.5 ng/10⁶ cells) ranging from 5.2 to 8.2 ng/10⁶ cells when cultured under hydrocortisone-free conditions. The amount of intracellular histamine substantially increased after exposure to hydrocortisone for 21 days and reached a plateau at the concentration of 1 μ M (Figure 3A). No change in the level of histamine was observed either in medium only or ethanol-supplemented cul-

Figure 4. Enhanced mRNA level of HDC in KU812 cells treated with hydrocortisone. After cultivation in medium only (None) or in the presence of 0.1% ethanol (EtOH) or 1 μ M hydrocortisone (HC), HDC and G3PDH mRNA were analyzed by RT-PCR. At certain time points (i.e., days 1, 3, 7, 14 and 21), 10 μ g of total RNA was reverse transcribed into cDNA. The resultant cDNA samples were subjected to 11 cycles of PCR for HDC and G3PDH. Southern blotting using specific probes was performed to confirm the PCR products. (A) Enhanced level of HDC mRNA in KU812 cells upon treatment with hydrocortisone for 21 days. Ten-fold serial dilutions (1:1, 1:10, 1:100) of cDNA were subjected to PCR. (B) Time-dependent elevation in the level of HDC mRNA in KU812 cells by hydrocortisone.

Figure 5. Induction of Fc ϵ RI expression on KU812 cells by hydrocortisone. (A) Overlay histograms represent the reactivity of KU812 cells with CRA1, human myeloma IgE or anti-human CD23 antibody. KU812 cells cultured in medium only (a, b, c) or in the presence of 0.1% ethanol (d, e, f) or 1 μ M hydrocortisone (g, h, i) for 21 days. Then, cells were incubated with mouse anti-human Fc ϵ RI monoclonal antibody CRA-1 (a, d, g) or human myeloma IgE (b, e, h) followed by staining with FITC-conjugated goat anti-mouse IgG or anti-human IgE respectively (hatched peaks). Mouse IgG2b (a, d, g) or human serum IgG (b, e, h) were used as isotype- or subclass-matched negative controls, respectively. During the same culture period, direct immunofluorescence was performed using either FITC-conjugated mouse anti-human CD23 antibody (hatched peak) or subclass-matched FITC-conjugated IgG1 antibody as a negative control (c, f, i). These immunostained samples were examined by flow cytometer. (B) Time-dependent enhancement of Fc ϵ RI expression on KU812 cells by hydrocortisone. Cells were cultivated in medium only (open circle) or in the presence of 0.1% ethanol (closed triangle) or 1 μ M hydrocortisone (closed circle). Cell surface expression of Fc ϵ RI was analyzed at the denoted time points depicted. The percentages of Fc ϵ RI positive cells were calculated with the arbitrary 1% cutoff channel position as determined by the negative control. Results are expressed as the mean $\pm$ SE (n = 5).

tures throughout the experiment (Figure 3B). KU812 cells treated with 1 μ M hydrocortisone for different periods of time showed a time-dependent increase in the intracellular histamine content, detectable at 3 to 7 days and increasing progressively up to 21 days (Figure 3B). More than a 10-fold increase (mean $\pm$ SE = 84.1 ± 4.0 ng/ 10^6 cells) was observed when KU812 cells were cultured with 1 μ M hydrocortisone for 21 days as compared with untreated cells. We next measured the mRNA level of HDC in KU812 cells by RT-PCR followed by Southern blotting (Figure 4). After treatment with 1 μ M hydrocortisone for 21 days, the cells showed an increase in the amount of HDC mRNA. The HDC mRNA taken from hydrocortisone-treated cells was detected clearly at a 1:100 dilution, whereas mRNA samples from both untreated and ethanol-treated cells were visualized only slightly (Figure 4A). In addition, hydrocortisone treatment resulted in a time-dependent elevation of the level of HDC mRNA in KU812 cells while no significant change was observed in the level of G3PDH during 21 day cultivation (Figure 4B). Thus, KU812 cells treated with hydrocortisone showed an increase in the expression of HDC mRNA, consistent with the increased amount of intracellular histamine.

Enhancement of cell surface expression of Fc ϵ RI on hydrocortisone-treated KU812 cells

Since cell surface expression of Fc ϵ RI was previously shown to be up-regulated during basophil differentiation, we examined the effect of hydrocortisone on the expression of Fc ϵ RI in KU812 cells. Fc ϵ RI is a tetrameric structure composed of one α -chain, one β -chain and two disulfide-linked γ -chains (Miller et al., 1989; Ra et al., 1989; Ravetch and Kinet, 1991). The Fc ϵ RI α -chain is a specific component of Fc ϵ RI, since the chain extends into the extracellular space and contains the entire IgE-binding site (Hakimi et al., 1990; Blank et al., 1991). As shown in Figure 5, flow cytometric analysis revealed that hydrocortisone enhanced the expression of Fc ϵ RI. The staining pattern with anti-human Fc ϵ RI α -chain monoclonal antibody CRA1 showed that Fc ϵ RI was very low if not totally absent on the cell surface (<1.0% of total cells) when the cells were cultured in hydrocortisone-free medium. After cultivation with 1 μ M hydrocortisone for 21 days, cell surface expression of Fc ϵ RI was induced (Figure 5A). Similar results were observed in five independent experiments. The expression of Fc ϵ RI was significantly enhanced at day 3, and further gradually

increased in a time-dependent manner until reaching a maximum (mean $\pm$ SE = $13.8\pm 4.0\%$) at day 21 (Figure 5B). Consistent with the data obtained using the CRA1 antibody, the binding pattern with human myeloma IgE indicated that hydrocortisone-treated KU812 cells were able to bind IgE on the cell surface at an apparently higher level than both non-treated and ethanol-treated cells (Figure 5A). To examine the possibility that this IgE binding to the cell surface was due to enhancement of CD23 expression, which is the low affinity IgE receptor Fc ϵ RII, flow cytometric analysis using anti-CD23 antibody was performed. CD23 expression was barely if at all detected when cells were cultured with or without hydrocortisone or with ethanol alone, suggesting that the ability of IgE to bind to hydrocortisone-treated KU812 cells was through Fc ϵ RI rather than Fc ϵ RII (Figure 5A). Taken together, hydrocortisone was able to induce expression of Fc ϵ RI in KU812 cells.

Histamine release from hydrocortisone-treated KU812 cells

The release of various mediators, including histamine, stored in cytoplasmic granules is a characteristic and critical function of basophils. It is well recognized that these cells release histamine in response to an elevation of the concentration of cytosolic free Ca^{2+} resulting from IgE-mediated stimuli or other non-IgE-mediated stimuli such as calcium ionophore A23187. We assessed the ability of histamine release from KU812 cells treated with or without hydrocortisone for 21 days upon stimulation with A23187 or human IgE/anti-IgE (Figure 6). In response to stimulation with A23187, non-treated and ethanol-treated cells showed 5.7 ± 0.2 ng/ 10^6 cells and 5.2 ± 0.4 ng/ 10^6 cells of histamine release, respectively. Under these hydrocortisone-free culture conditions, the amount of histamine released when stimulated with human IgE/anti-IgE stimulation was as low as non-stimulated cells. However, when cells were cultivated with 1 μ M hydrocortisone, stimulation with not only the A23187 but also with human IgE/anti-IgE resulted in the release of histamine with values of 68.7 ± 2.6 ng/ 10^6 cells (about 88% of intracellular content) or 26.5 ± 2.4 ng/ 10^6 cell (about 34% of intracellular content), respectively. These results suggest that KU812 cells treated with hydrocortisone became basophil-like enough in function to be able to release histamine. In addition, it is likely that the hydrocortisone-induced Fc ϵ RI molecules on the cell

Figure 6. Histamine release from KU812 cells. KU812 cells cultivated with or without 1 μ M hydrocortisone or 0.1% ethanol for 21 days were stimulated with Ca^{2+} ionophore A23187 or mouse anti-human IgE antibody after sensitization with human myeloma IgE at 37 $^{\circ}\text{C}$ for 20 min. Results are expressed as the mean $\pm$ SE ($n = 5$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (by Student's t-test).

surface are able to transduce the appropriate intracellular signals to result in degranulation.

Discussion

While glucocorticoids are useful for their immunosuppressive and anti-inflammatory action, these agents have pleiotropic effects on hematopoietic cells. Glucocorticoids bind to and activate the glucocorticoid receptors, which induce apoptosis in lymphoid and myelomonocytic cells, even though they protects granulocytes from apoptosis (Liles et al., 1995), and stimulates proliferation of erythroid progenitors (Wessely et al., 1997). We evaluated the efficacy of hydrocortisone on human leukemia KU812 cells, which have been described as pre-basophilic lineage cells. As shown in this study, cell growth and viability of KU812 cells were not affected by hydrocortisone. It is likely that hydrocortisone has no effect on induction of apoptosis in KU812 cells. Previous studies revealed

that KU812 cells exhibit a spontaneous capacity to differentiate into mature hemopoietic cells including basophils (Fukuda et al., 1987; Nakazawa et al., 1989). Throughout our experiment, morphologically mature basophilic cells expressing cytoplasmic granules which are stained metachromatically with Wright dye or toluidine blue dye were found at a very low percentage when KU812 cells were cultured without hydrocortisone. Treatment with 1 μ M hydrocortisone resulted in neither significant increase nor decrease in the percentage of granulated cells. Although staining analysis gave no information as to selective proliferation or differentiation of basophilic cells, we found that hydrocortisone is able to increase the amount of intracellular histamine and up-regulate the expression of HDC mRNA in KU812 cells. Among blood cell types, basophils and mast cells are the only cell types that can synthesize histamine, which is catalyzed from L-histidine by HDC (Krauss et al., 1968; Maeyama et al., 1988). It has been reported that histamine synthesis is promoted and up-regulated during the dif-

ferentiation of basophils (Valent et al., 1989). Our data suggest that HDC molecules increase in proportion to the level of HDC mRNA, by which histamine synthesis is up-regulated. Therefore, the gradual constant increase in the amount of histamine for up to 21 days implies that the cells underwent further differentiation to become basophilic cells in the presence of hydrocortisone.

Along with the increase in the amount of intracellular histamine, flow cytometric analysis revealed that hydrocortisone treatment resulted in an enhancement of Fc ϵ RI expression on the cell surface. In basophils and mast cells, it has been previously shown that IgE and IL-4 are factors that can induce Fc ϵ RI expression. IgE binding to surface Fc ϵ RI is thought to stabilize the receptor complexes, which, in turn, facilitate an accumulation of Fc ϵ RI on the cell surface (MacGlashan et al., 1999). IL-4 has been reported to up-regulate the amount of Fc ϵ RI α -chain mRNA, which contributes to the enhancement of Fc ϵ RI expression (Toru et al., 1996). Since our data raises the possibility that hydrocortisone has an ability to facilitate the *in vivo* expression of Fc ϵ RI on the surface of basophils and/or mast cells, it is necessary to examine its effect on transcription as well as the protein levels of each subunit, Fc ϵ RI α , β , γ -chain. Whatever the expression mechanism is, the induced Fc ϵ RI molecules has been shown to bind IgE and to be functional with regard to the transmission of the degranulation signal in response to IgE/anti-IgE antibody stimulation. A critical function of basophils in the allergic reaction is the release of inflammatory mediators such as histamine, proteases and chemotactic factors when allergen cross-links the specific IgE binding to Fc ϵ RI on the surface of the cells. It seems likely that hydrocortisone accelerates the differentiation of KU812 cells to obtain the features and function of basophils although the morphological character is not fully acquired. Although KU812 cells are of leukemic origin, our data would imply that hydrocortisone, an endogenous glucocorticoid, may be, if not entirely, responsible for the *in vivo* differentiation of certain precursor cells into basophils.

Gene expression conferring certain specific phenotypes during the differentiation process is often regulated by lineage-specific transcription factors. In mast cells, the expression of MC-CPA, a mast cell specific protease, is known to be regulated by the GATA-1 transcription factor, which is a member of GATA-binding protein family (Zon et al., 1991). GATA-1 was originally isolated for its ability to bind to the

GATA site present in the promoters and enhancers of many erythroid genes responsible for normal erythroid development (Plumb et al., 1986; Wall et al., 1988; Trainor and Engel, 1989; Tsai et al., 1989; Mignotte et al., 1989; Zon et al., 1991). Later, it became evident that basophils and mast cells express GATA-binding proteins including GATA-1, GATA-2 (Zon et al., 1993; Henkel and Brown, 1994). While the relationship between the expression of MC-CPA activated by GATA-1 and differentiation of mast cells is suspected, it has been reported that the level of MC-CPA mRNA increases upon treatment with dexamethasone, a synthetic glucocorticoid, in mBMMC (Eklund et al., 1997). There is an evidence that four GATA consensus sequences are present in the promoter region of the human HDC gene (Yatsunami et al., 1994). It has been recently shown that GATA-1 binds to enhancers of human Fc ϵ RI α -chain gene (Nishiyama et al., 1999). In this study, we demonstrated that hydrocortisone is able to increase the amount of HDC mRNA and enhance cell surface expression of Fc ϵ RI in KU812 cells. Accordingly, our results raise the possibility that glucocorticoids may enhance the expression of HDC and Fc ϵ RI α -chain genes through activation or expression of GATA-binding proteins and that the proteins are important to basophil-specific gene expression, leading to an exhibition of basophil function. The mechanisms by which glucocorticoids affect the expression of HDC and Fc ϵ RI α -chain genes remain to be elucidated.

To our knowledge, this is the first study to report that glucocorticoids can act to trigger a differentiation process of human basophils. These results shown here may confer yet another novel role for glucocorticoids in the immune system.

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