

Short communication

Expression of adhesion molecules on cord-blood-derived, cultured human mast cells and effect of dexamethasone on intercellular adhesion molecule-1 expression on the mast cells treated by phorbol myristate acetate

Background: Increase in mast-cell number at sites of allergic inflammation has been observed, and glucocorticoids applied to the sites have been shown to result in a significant reduction in mast cells. However, the expression of adhesion molecules on cultured human mast cells and their regulation by glucocorticoids is poorly understood.

Methods: Cultured human mast cells were raised from human umbilical cord-blood cells, and the expression of adhesion molecules on the mast cells was analyzed by flow cytometry. The cells were also incubated with 10 ng/ml phorbol myristate acetate (PMA) for the indicated time, and the effect of dexamethasone on adhesion molecule expression on PMA-treated, cultured human mast cells was examined.

Results: Cord-blood-derived, cultured human mast cells constitutively expressed intercellular adhesion molecule-1 (ICAM-1), very late antigen-4 (VLA-4), and macrophage-1 antigen (Mac-1). Weak expression of lymphocyte function-associated antigen-1 (LFA-1) was observed on the cells, whereas they failed to express vascular cell adhesion molecule-1 (VCAM-1). Kinetic studies showed that after a transient downregulation reaching a minimum at 8 h, the expression of ICAM-1 was markedly upregulated on PMA-treated mast cells after a 24-h incubation. In contrast, the expression of VLA-4 and Mac-1 was decreased after the incubation with PMA for 24 h. The PMA-induced upregulation of ICAM-1 was inhibited by dexamethasone in a concentration-dependent manner.

Conclusions: Our results indicate that cord-blood-derived, cultured human mast cells constitutively express integrins and ICAM-1, but not VCAM-1, and demonstrate for the first time that dexamethasone inhibits the upregulation of ICAM-1 on PMA-treated, cultured human mast cells.

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Mast cells originate in the bone marrow, and reside in connective tissue and at the mucosal surface. However, little is known about the factors involved in the homing of mast cells into tissues. It is reasonable to propose that circulating committed mast-cell progenitors first adhere to endothelial cells, and then migrate through tissues to specific locations. Moreover, increase in mast-cell number at sites of allergic inflammation has been observed (1), suggesting that adhesion molecules expressed on mast cells may be involved in the mast-cell migration into inflamed tissues (2). On the other hand, glucocorticoids are widely used for the treatment of allergic

diseases, and glucocorticoids applied to the skin (3) and lung (4) have been shown to result in a significant reduction in mast cells.

Investigations of the expression of adhesion molecules on mast cells have been performed, largely in the murine system with bone-marrow-derived mast cells (5). In analogy to murine bone-marrow-derived mast cells, rat peritoneal mast cells have also been shown to express several adhesion molecules (6). The problem is that mast cells are heterogeneous, and there may be functional differences in mast cells between different species.

In the human system, very late antigen-4 (VLA-4

or CD49d/CD29), VLA-5 (CD49e/CD29), and intercellular adhesion molecule-1 (ICAM-1 or CD54) have been found to be expressed on mast cells from the heart, uterus, lung, and skin (7–10). The human leukemic mast-cell line HMC-1 has also been used to study the expression of adhesion molecules, and this cell line has been shown to express lymphocyte function-associated antigen-1 (LFA-1 or CD11a/CD18) and ICAM-1, while expression of macrophage-1 antigen (Mac-1, complement receptor 3 or CD11b/CD18) and protein 150,95 (p150,95, complement receptor 4 or CD11c/CD18) is low (11, 12). However, HMC-1 cells lack high-affinity surface receptors for IgE (FcεRI) (13), and are immature mast cells which contain 1% of the tryptase of isolated lung mast cells.

Recently, it became possible to obtain cultured human mast cells from umbilical cord-blood cells (14, 15). Their advantages are that relatively large numbers of the cells are accessible, and that alterations of surface membrane which might occur during the purification process of mast cells from tissues can be avoided. Nevertheless, regulation of adhesion molecules on cord-blood-derived, cultured human mast cells is not fully understood. Toru et al. (16) found that cord-blood-derived, cultured human mast cells expressed LFA-1 and ICAM-1, but not VLA-2 (CD49b/CD29) or vascular cell adhesion molecule-1 (VCAM-1 or CD108). However, Tachimoto et al. (17) have reported that cultured human mast cells expressed ICAM-1 and β integrins including VLA-2, and Ochi et al. (18) have shown the expression of β_3 integrin on cultured human mast cells during their maturation. However, investigations of the regulation of adhesion molecules on cultured human mast cells by glucocorticoids have never been reported. In fact, the effect of glucocorticoids on cultured human mast cells has been little reported, although antiasthma drugs, such as disodium cromoglycate and β -agonists, are shown to inhibit mediator release from cord-blood-derived, cultured human mast cells (19).

In the present study, we first examined the expression of various surface adhesion molecules on cord-blood-derived, cultured human mast cells, and, second, the kinetics of adhesion molecules on phorbol myristate acetate (PMA)-treated, cord-blood-derived, cultured human mast cells. Finally, we investigated the effect of dexamethasone on ICAM-1 expression on PMA-treated, cord-blood-derived, cultured human mast cells. Our results indicate that cultured human mast cells constitutively express integrins and ICAM-1, but not VCAM-1, and demonstrate for the first time the effect of glucocorticoids on cord-blood-derived, cultured human mast cells. We showed that dexamethasone inhibits the upregulation of ICAM-1 on PMA-treated, cultured human mast cells, indicat-

ing that regulation of adhesion molecules on human mast cells may be a part of the anti-inflammatory effect of glucocorticoids in the treatment of allergic diseases.

Material and methods

Human mast-cell culture

Mast cells were raised from human umbilical cord blood cells by a previously described method (14, 15). Briefly, freshly obtained heparin-treated umbilical cord blood (about 20 ml) was layered over Ficoll-Paque (Pharmacia & Upjohn, Uppsala, Sweden), and centrifuged at 400 g for 20 min. The mononuclear cell layer was collected and suspended at 1×10^6 cells/ml in Media I, which was a mixture of Dulbecco's modified Eagle's medium component plus 10 μ g/ml insulin, 10 μ g/ml transferrin, 5×10^{-5} M 2-mercaptoethanol, 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, and 2.6 ng/ml NaSeO₃ (IBL Men-eki Seibutsu Kenkyujo, Gunma, Japan), supplemented by 5% fetal bovine serum (Cytosystems, Castle Hill, Australia), 50 ng/ml human recombinant interleukin 6 (IL-6), and 80 ng/ml human recombinant stem-cell factor (SCF) (PeproTech, Rocky Hill, NJ, USA) in a 37°C CO₂ (5%) humidified incubator. The cells were passaged every week and maintained in the same medium. Cell counting was performed by toluidine blue staining. Mast cells were then fixed with Carnoy's fixative for 15 min at room temperature and stained for mast-cell tryptase and chymase with monoclonal murine antitryptase and antichymase antibodies (Chemicon, Temecula, CA, USA). Aliquots of the cells were removed for histamine assay and flow cytometry assay. This study complied with the Review Board of Akita University School of Medicine (Japan).

Monoclonal antibodies (mAbs) and flow cytometry

To characterize the pattern of selected surface molecules, we performed single-color immunofluorescence on mast cells stained indirectly with the following mAbs: antihuman VLA-4, antihuman ICAM-1 (Immunotech, Marseille, France); antihuman LFA-1 α (CD11a), antihuman LFA-1 β (β_2 -chain or CD18), antihuman VCAM-1 (Dako, Glostrup, Denmark); and antihuman Mac-1 (Nichirei, Tokyo, Japan). The fluorescein isothiocyanate (FITC)-conjugated F(ab')₂ fragment of goat antimouse IgG was provided by Tago, Camarillo, CA, USA. The isotype of all mAbs was IgG₁, and mouse IgG₁ (Dako) was used as a subclass-matched primary control antibody. PMA and dexamethasone were purchased from Sigma, St Louis, MO, USA.

For analysis, 1×10^5 cells in a volume of 100 μ l were incubated with 1 μ g of the primary mAb or the equivalent amount of a mouse isotype control antibody in the dark for 30 min at 4°C, washed twice with ice-cold phosphate-buffered saline (PBS) (containing 0.1% [w/v] bovine serum albumin, 0.1% [w/v] sodium azide), and conjugated with the second FITC-conjugated F(ab')₂ fragment of goat antimouse IgG (dilution 1:100) in the dark for 30 min at 4°C. The cells were washed twice more and resuspended in PBS before analysis by FACScan (Becton Dickinson, Mountain View, CA, USA). Nonviable cells were gated out with the window, and at least 5000 events were analyzed. The mean fluorescent intensity (MFI), which was considered to correlate roughly with surface adhesion molecule density, was measured, and background antibody binding was estimated with an isotype-matched negative control mAb. The ratio of MFI was calculated by estimating the MFI of each control at 1.

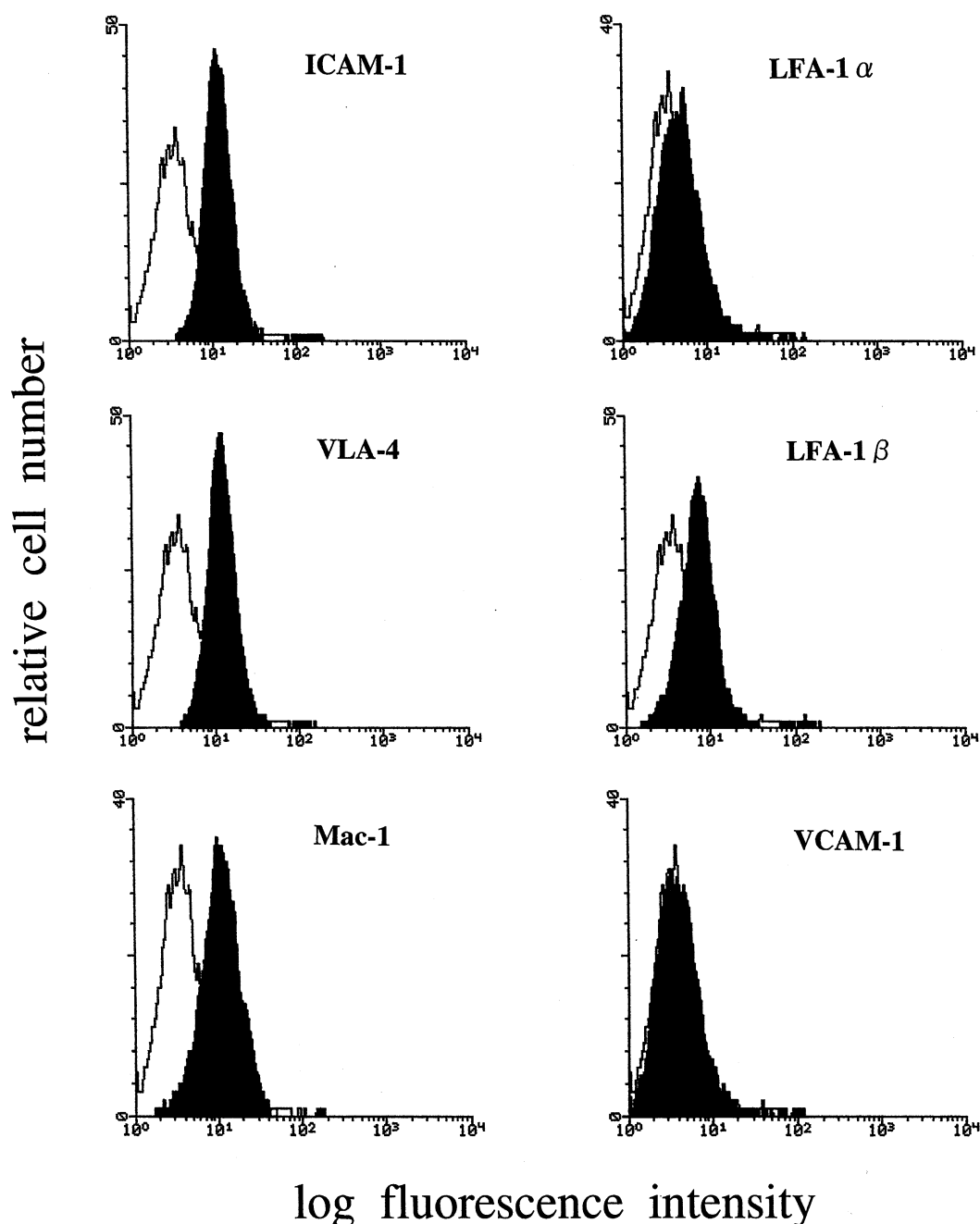


Figure 1. Adhesion molecules expressed on cord-blood-derived, cultured human mast cells. Representative experiment is shown (open portion: subclass-matched control antibody; shaded portion: mouse mAb specific for adhesion molecule). Very similar results were obtained in four independent experiments at each time under same conditions.

Stimulation of cells

Human mast cells were plated in culture dishes, and were incubated in the presence of 80 ng/ml SCF and 50 ng/ml IL-6 in a 37°C CO₂ (5%) humidified incubator. The cells were harvested for up to 24 h in the absence or presence of PMA at a concentration of 10 ng/ml, as reported by Weber et al. (11). This time of incubation was selected on the basis of preliminary time-response curves, demonstrating optimal modulation of the expression of the investigated markers in the absence of significant cell death, as assessed by the trypan blue exclusion test. Control cells were kept at 37°C in the absence of the stimulus for the indicated times. In other experiments, the cells were preincubated with different concentrations of dexamethasone for

30 min and subsequently incubated with PMA, and were assessed for expression of ICAM-1 by flow cytometry. Before the flow cytometric analysis, cell viability more than 98% was confirmed by the trypan blue method.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed by paired two-way Student's *t*-test. The effect of dexamethasone on the expression of ICAM-1 was determined by analysis of variance (ANOVA) followed by a Bonferroni *t*-test. A *P* value of <0.05 was considered statistically significant.

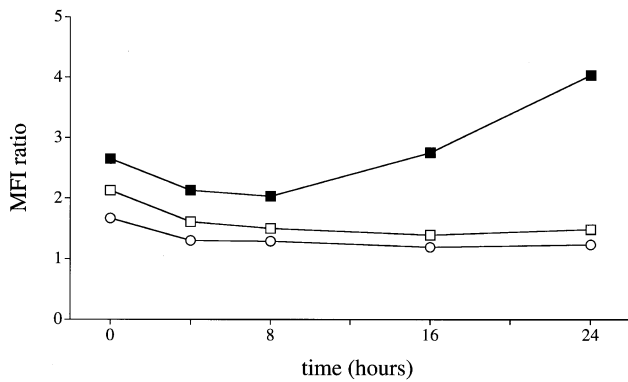


Figure 2. Kinetics of adhesion molecules expressed on PMA-treated, cord-blood-derived, cultured mast cells. Cord-blood-derived, cultured human mast cells were incubated with PMA at concentration of 10 ng/ml for indicated time. Representative experiment is shown. Very similar results were obtained in three independent experiments at each time under same conditions. ■: ICAM-1; □: VLA-4; ○: Mac-1.

Results

The purity of mast cells reached virtually 100% after 10 weeks; in the present study, we used cells which were more than 100 days old. At the time of the study, $99.0 \pm 1.4\%$ (mean $\pm$ SEM, $n=9$) of the cells were trypan blue negative and $98.5 \pm 3.2\%$ toluidine blue positive ($n=9$). When the cells were immunocytochemically stained for tryptase, $99.2 \pm 2.8\%$ were positively stained ($n=9$). Among tryptase-positive cells, $14.2 \pm 2.9\%$ were weakly immunocytochemically stained for chymase ($n=9$). Nine different cultures of mast cells were used in this study.

We first examined the expression of various surface

adhesion molecules on cord-blood-derived, cultured human mast cells. Representative experiments demonstrating the constitutive expression of ICAM-1, VLA-4, and Mac-1 on cord-blood-derived, cultured human mast cells are shown in Fig. 1. Weak expression of LFA-1 α and LFA-1 β was observed on the cells, whereas they failed to express VCAM-1. The MFI ratio of ICAM-1, VLA-4, Mac-1, LFA-1 α , LFA-1 β , and VCAM-1 was 2.68 ± 0.30 (mean $\pm$ SEM, $n=4$), 2.43 ± 0.37 , 2.07 ± 0.36 , 1.37 ± 0.02 , 1.56 ± 0.14 , and 1.01 ± 0.04 , respectively. Next, PMA at a concentration of 10 ng/ml was added to other mast-cell cultures, and the time kinetic studies were done up to 24 h because of reduced viability thereafter. As shown in Fig. 2, after a transient downregulation reaching a minimum at 8 h, the expression of ICAM-1 was markedly upregulated on PMA-treated mast cells after a 24-h incubation. The MFI ratios of ICAM-1, VLA-4, and Mac-1 after 24-h incubation with PMA or medium as control were 3.27 ± 0.25 and 2.61 ± 0.07 ($n=5$), 1.63 ± 0.07 and 2.34 ± 0.08 , and 1.41 ± 0.07 and 1.87 ± 0.11 , respectively. The expression of ICAM-1 after a 24-h incubation with PMA was significantly increased ($P < 0.01$). In contrast, the expression of VLA-4 and Mac-1 on the cells was significantly decreased after the incubation with PMA ($P < 0.01$). Finally, the effect of dexamethasone on PMA-induced increase of ICAM-1 expression on cultured human mast cells was investigated. Dexamethasone at concentrations from 0.01 to 1 μ M was found to have no significant effect on the constitutive expression of ICAM-1 on cultured human mast cells (data not shown). Representative experiments showing the expression of ICAM-1 after a 24-h

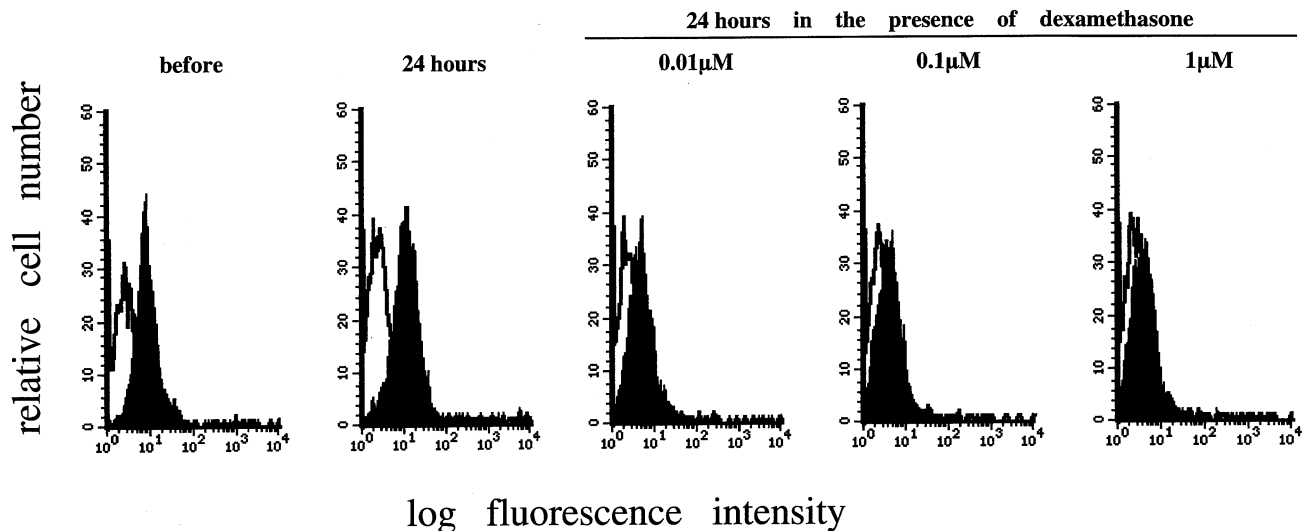


Figure 3. Increased expression of ICAM-1 on cord-blood-derived, cultured human mast cells after 24-h incubation with PMA and effect of dexamethasone on PMA-induced increased expression of ICAM-1. Cord-blood-derived, cultured human mast cells were pretreated in presence or absence of dexamethasone at concentrations indicated for 30 min and subsequently stimulated with PMA at concentration of 10 ng/ml for 24 h. Control cells were incubated for 24 h at 37°C in absence of stimulus and dexamethasone. Representative experiment is shown (open portion: subclass-matched control antibody; shaded portion: mouse mAb specific for ICAM-1). Very similar results were obtained in four independent experiments at each time under same conditions.

incubation with PMA and the effect of dexamethasone on the expression are shown in Fig. 3. The MFI ratios of ICAM-1 on PMA-treated, cultured human mast cells in the absence or presence of dexamethasone at concentrations 0.01, 0.1, and 1 μ M were 3.42 ± 0.07 ($n=4$), 2.01 ± 0.07 , 1.85 ± 0.03 , and 1.65 ± 0.04 , respectively. The PMA-induced upregulation of ICAM-1 was inhibited significantly by dexamethasone at each concentration ($P < 0.01$). The MFI ratio of ICAM-1 expressed on PMA-treated mast cells in the presence of dexamethasone at each concentration was significantly decreased compared with the MFI ratio of the control (2.66 ± 0.11) ($P < 0.01$), and the ratio in the presence of dexamethasone at a concentration of 1 μ M was significantly lower than that in the presence of dexamethasone at a concentration of 0.01 μ M ($P < 0.01$).

Discussion

Recent advances of science led us to obtain cultured human mast cells raised from umbilical cord-blood cells. Their advantages are that relatively large numbers of the cells are accessible, and that alterations which might occur during the purification process from tissues can be avoided. In fact, these cells are histamine-containing, IgE receptor-positive, and metachromatic (14, 15).

Although it is generally accepted that mast-cell precursors are derived from bone-marrow progenitors migrating in the circulation prior to entering tissues where they terminate their differentiation, the mechanisms whereby immature mast cells leave the bloodstream in normal development and under inflammatory conditions remain poorly understood. However, studies on the emigration of other leukocytes from the circulation in response to inflammatory stimuli have revealed striking similarities concerning the adhesion molecules involved in interaction with the endothelium (20), and it seems likely that adhesion molecules on mast cells may be involved in the process of migration and tissue distribution of mast cells (2).

The present study showed a detectable constitutive expression of ICAM-1, VLA-4, and Mac-1 on cord-blood-derived, cultured human mast cells, whereas the expression of LFA-1 α and LFA-1 β was low. Our cord-blood-derived, cultured human mast cells failed to express VCAM-1, and this finding was consistent with the previous data (15, 16). The finding of LFA-1 α and LFA-1 β expression in the present study differs from the data reported by Toru et al. (16), which showed strong constitutive expression of LFA-1 α and LFA-1 β on cord-blood-derived, cultured human mast cells. However, previous investigations indicated a lack of LFA-1 α and LFA-1 β expression on human mast cells purified from heart, lung, and skin (7, 9, 10), and

immature mast cells of the HMC-1 cell line have been shown to express β_2 -integrins and ICAM-1 (12). The precise reason for the difference between our observation and the previous report (16) is unknown; however, we used more than 100-day-old mast cells in the present study, and the difference may be due to the culture time of mast cells in the presence of SCF and IL-6.

All types of mast cells dispersed from human tissues and HMC-1 cells have been shown to express ICAM-1 on their surface (7-12), suggesting the possible importance of this adhesion molecule on human mast cells. The present study showed that after a transient downregulation reaching a minimum at 8 h, the expression of ICAM-1 was markedly upregulated on PMA-treated, cord-blood-derived, cultured human mast cells after a 24-h incubation. Since the upregulation was slow, it may require *de novo* protein synthesis in the cells, as observed in HMC-1 cells (12). In contrast, the expression of VLA-4 and Mac-1 was decreased after the incubation with PMA for 24 h. The mechanism responsible for downregulation may involve internalization, proteolytic cleavage of the molecules from the cell surface, or a conformational alteration, resulting in loss or decrease in avidity of the epitopes recognized by the antibodies used. Although we could not demonstrate it in the present paper, shedding may be the mechanism responsible for downregulation since neutral proteases might be released from the activated mast cells. The mechanism for the downregulation and its physiologic relevance remains unknown so far, and further studies are required.

Accumulation of mast cells at sites of allergic inflammation (1), together with T cells or eosinophils, has been demonstrated particularly in allergic rhinitis (21), atopic dermatitis (22), and bronchial asthma (23). Therefore, surface adhesion molecules on mast cells may mediate a cell-contact-dependent regulatory mechanism of mast-cell interaction with these effector cells. On the other hand, the local delivery of glucocorticoids to tissues has been shown to decrease mast-cell numbers in allergic rhinitis (24), immediate dermal allergic reaction (3), and bronchial asthma (4), and this pharmacologic effect is believed to be one of the mechanisms by which glucocorticoids regulate allergic inflammation.

Although glucocorticoids have been shown to suppress the expression of adhesion molecules on endothelial cells (25), their effect on adhesion molecules on cord-blood-derived, cultured human mast cells has never been reported. The present study showed for the first time that the upregulation of ICAM-1 on PMA-treated, cord-blood-derived, cultured human mast cells was inhibited by dexamethasone in a concentration-dependent manner. ICAM-1 expression on PMA-treated human mast cells was significantly decreased by dexamethasone at

the concentrations investigated as compared with the constitutive control level. In fact, differentially induced ICAM-1 expression has been shown to be decreased upon exposure to dexamethasone in several cell systems (25), and phorbol ester-induced upregulation of CD18 has been shown to be decreased upon dexamethasone treatment in a human monocytic cell line (26). Dexamethasone has also been shown to alter the cell-surface density of β_2 -integrins and ICAM-1 on HMC-1 cells (27). Therefore, the decreased expression of ICAM-1 on human mast cells caused by glucocorticoids may result in the suppression of the infiltration of mast cells in the treatment of allergic diseases. However, our observations are limited to cord-blood-derived, cultured human mast cells treated with PMA; functional analysis of ICAM-1 expression was not performed

in the present study. These points raise some questions about their biologic relevancy, and further experiments using specific stimulation are required.

In conclusion, we have found that cord-blood-derived, cultured human mast cells constitutively express integrins and ICAM-1, but not VCAM-1, suggesting that these adhesion molecules might play a key role in the migration of mast-cell precursors into tissues in normal physiology and under pathologic conditions. We also demonstrate for the first time that dexamethasone inhibits the upregulation of ICAM-1 on PMA-treated, cultured human mast cells, indicating that regulation of ICAM-1 on human mast cells may be a part of the anti-inflammatory effect of glucocorticoids in the treatment of allergic diseases.

References

- ISHIZAKA T, ISHIZAKA K. Biology of immunoglobulin E. Molecular basis of reagenic hypersensitivity. *Prog Allergy* 1975;**19**:60–121.
- HAMAWY MM, MERGENHAGEN SE, SIRAGANIAN RP. Adhesion molecules as regulators of mast-cell and basophil function. *Immunol Today* 1994;**15**:62–66.
- PIPKORN U, HAMMARLUND A, ENERBÄCK L. Prolonged treatment with topical glucocorticoids results in an inhibition of the allergen-induced wheal and flare response and a reduction in skin mast cell numbers and histamine content. *Clin Exp Allergy* 1989;**19**:19–25.
- DUDDRIDGE M, WARD C, HENDRICK DJ, WALTERS EH. Changes in bronchoalveolar lavage inflammatory cells in asthmatic patients treated with high dose inhaled beclomethasone dipropionate. *Eur Respir J* 1993;**6**:489–497.
- DASTYCH J, METCALFE DD. Stem cell factor induces mast cell adhesion to fibronectin. *J Immunol* 1994;**152**:213–219.
- GURISH MF, BELL AF, SMITH TJ, DUCHARME LA, WANG RK, WEIS JH. Expression of murine β_7 , α_4 , and β_1 integrin genes by rodent mast cells. *J Immunol* 1992;**149**:1964–1972.
- SPERR WR, BANKL HC, MUNDIGLER G, et al. The human cardiac mast cell: localization, isolation, phenotype, and functional characterization. *Blood* 1994;**84**:3876–3884.
- GUO CB, KAGEY-SOBOTKA A, LICHTENSTEIN LM, BOCHNER BS. Immunophenotyping and functional analysis of purified human uterine mast cells. *Blood* 1992;**79**:708–712.
- VALENT P, ASHMAN LK, HINTERBERGER W, et al. Mast cell typing: demonstration of a distinct hematopoietic cell type and evidence for immunophenotypic relationship to mononuclear phagocytes. *Blood* 1989;**73**:1778–1785.
- LAWRENCE ID, WARNER JA, COHAN VL, HUBBARD WC, KAGEY-SOBOTKA A, LICHTENSTEIN LM. Purification and characterization of human skin mast cells: evidence for human mast cell heterogeneity. *J Immunol* 1987;**139**:3062–3069.
- WEBER S, RUH B, KRÜGER-KRASAGAKES S, CZARNETZKI BM. Adhesion molecules on the human mast cell line HMC-1 are up-regulated during cell activation. *Arch Dermatol Res* 1995;**287**:695–698.
- WEBER S, BABINA M, FELLER G, HENZ BM. Human leukaemic (HMC-1) and normal skin mast cells express β_2 -integrins: characterization of β_2 -integrins and ICAM-1 on HMC-1 cells. *Scand J Immunol* 1997;**45**:471–481.
- NILSSON G, BLOM T, KUSCHE-GULLBERG M, et al. Phenotypic characterization of the human mast cell line HMC-1. *Scand J Immunol* 1994;**39**:489–498.
- IGARASHI Y, KUROSAWA M, ISHIKAWA O, et al. Characteristics of histamine release from cultured human mast cells. *Clin Exp Allergy* 1996;**26**:597–602.
- SAITO H, EBISAWA M, TACHIMOTO H, et al. Selective growth of human mast cells induced by steel factor, IL-6, and prostaglandin E_2 from cord blood mononuclear cells. *J Immunol* 1996;**157**:343–350.
- TORU H, KINASHI T, RA C, NONOYAMA S, YATA J, NAKAHATA T. Interleukin-4 induces homotypic aggregation of human mast cells by promoting LFA-1/ICAM-1 adhesion molecules. *Blood* 1997;**89**:3296–3302.
- TACHIMOTO H, HUDSON SA, BOCHNER BS. Acquisition and alteration of adhesion molecules during cultured human mast cell maturation [Abstract]. *J Allergy Clin Immunol* 2000;**105**:S85.
- OCHI H, HIRANI WM, YUAN Q, FRIEND DS, AUSTEN KF, BOYCE JA. T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells *in vitro*. *J Exp Med* 1999;**190**:267–280.
- SHICHIO M, INAGAKI N, NAKAI N, et al. The effects of anti-asthma drugs on mediator release from cultured human mast cells. *Clin Exp Allergy* 1998;**28**:1228–1236.
- SPRINGER TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;**76**:301–314.
- BENTLEY AM, JACOBSON MR, CUMBERWORTH V, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992;**89**:877–883.
- LEUNG DY. Role of IgE in atopic dermatitis. *Curr Opin Immunol* 1993;**5**:956–962.
- BRADDING P, ROBERTS JA, BRITTEN KM, et al. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994;**10**:471–480.

24. JACOBSON MR, JULIUSSON S, LÖWHAGEN O, BALDER B, KAY AB, DURHAM SR. Effect of topical corticosteroids on seasonal increases in epithelial eosinophils and mast cells in allergic rhinitis: a comparison of nasal brush and biopsy methods. *Clin Exp Allergy* 1999;**29**:1347–1355.
25. CROSTEIN BN, KIMMEL SC, LEVIN RI, MARTINIUK F, WEISSMANN G. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc Natl Acad Sci U S A* 1992;**89**:9991–9995.
26. HOFF T, SPENCKER T, EMMENDOERFFER A, GOPPELT-STRUEBE M. Effects of glucocorticoids on the TPA-induced monocytic differentiation. *J Leukoc Biol* 1992;**52**:173–182.
27. BABINA M, WEBER S, HENZ BM. Retinoic acids and dexamethasone alter cell-surface density of β_2 -integrins and ICAM-1 on human leukemic (HMC-1) mast cells. *Arch Dermatol Res* 1997;**289**:111–115.