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Glucocorticoid Suppresses Autocrine Survival of Mast Cells by Inhibiting IL-4 Production and ICAM-1 Expression

Hideshi Yoshikawa, Yasuo Nakajima, and Kachio Tasaka

When mast cells are activated through their high affinity IgE receptors (FcerI), release of chemical mediators is followed by secretion of multiple cytokines. In this work, we report that IL-3-dependent mast cell line MC9 undergoes apoptosis when IL-3 is withdrawn. However, cross-linking of FcerI prevents apoptosis of MC9 by an autocrine mechanism, producing IL-3, IL-4, and GM-CSF. Although stimulated MC9 synthesizes mRNAs and proteins of these cytokines, secretion of endogenous IL-3 and GM-CSF is not enough for cell survival, whereas IL-4 itself does not have survival effect on MC9, but it induces cell aggregation by expressing LFA-1 and makes it reactive to endogenous growth factors. Addition of dexamethazone (DXM) to MC9 results in significant down-regulation of IL-4 mRNA in activated MC9. However, mRNA levels of IL-3 and GM-CSF are not changed by DXM. DXM also directly down-regulates the expression of ICAM-1 that is the high affinity ligand of LFA-1, by which the self-aggregation of MC9 is inhibited. Thus, glucocorticoids suppress autocrine survival of mast cells by inhibiting IL-4 production and ICAM-1 expression. The Journal of Immunology, 1999, 162: 6162–6170.

Mast cells are widely distributed throughout vascularized tissues. They represent a source of potent mediators of inflammation. These mediators are released after sensitization with IgE, which are bound to FcerI on mast cells. Such activation causes mast cells to degranulate, releasing chemical mediators. Activated mast cells also elaborate newly synthesized mediators such as products of arachidonic acid metabolism. Mast cells are regarded as critical effector cells in the inflammatory reactions of IgE-dependent immediate hypersensitivity. However, glucocorticoids (GCS), which are effective against allergic inflammation, suppress neither IgE production nor the release of chemical mediators from mast cells. Mast cells produce multiple cytokines, including IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, TNF-α, and GM-CSF, by cross-linking of FcerI (1–6). These cytokines induce infiltration of inflammatory cells such as CD4+ T cells and eosinophils after immediate response. These phenomena are called late responses, which are effectively suppressed by GCS (7).

One population of mast cells, referred to as mucosal mast cells, appears to be extremely sensitive to Th2-associated cytokines IL-3 and IL-4. IL-3 is the major cytokine regulating proliferation of these subsets, whereas in vitro studies indicate that IL-4 acts as a costimulant of proliferation (8). IL-4 also triggers and supports proliferation of connective tissue-type mast cells synergistically with IL-3 (9). In addition, some unstimulated IL-3-dependent mast cell lines transcribe low levels of IL-4 mRNA, but do not secrete detectable levels of IL-4 (1). Clinically, in atopic patients, increased numbers of cells positive for IL-4 mRNA have been found during allergen-induced late-phase cutaneous response, suggesting IL-4 is up-regulated in human allergic diseases (10). Moreover, allergen immunotherapy decreases IL-4 production in CD4+ T cells from allergic individuals (11, 12). Dysregulation of IL-4 gene expression is also reported in steroid-resistant asthma (13). In vivo, Abs to IL-3 and IL-4 have been shown to suppress helminth-induced intestinal mastocytosis (14).

Thus, IL-4 is a multifunctional cytokine that appears to play an important role in the pathogenesis of allergic disease. In vivo IgE responses in mice are inhibited after prior administration of neutralizing anti-IL-4 Abs (15). IL-4-deficient mice do not develop IgE responses (16, 17), whereas transgenic mice with enhanced IL-4 production develop high circulating IgE levels and severe chronic conjunctivitis, characterized by mononuclear cells, mast cells, and eosinophil infiltration (18). These cellular changes are similar to those observed in human allergic asthma, rhinitis, dermatitis, and keratoconjunctivitis. Both human and rodent T cells of the Th2 phenotype have been shown to produce this cytokine, but they first require the presence of IL-4 for their own development (19, 20). It is our hypothesis that in an allergic response, this signal may come from the mast cells. In support of this, IL-4 generation has also been shown by rodent (3) and human (21) mast cells, and non-B non-T cells (22, 23), which have characteristics of both mast cells and basophils.

In this way, these cytokines play a critical role in late-phase reaction in allergic inflammation. Therefore, we investigated the mechanism of mast cell survival by using exogenous and endogenous cytokines induced by cross-linking of FcerI. In addition, we studied the direct effects of GCS on mast cells. This study suggests a model for the local interaction of mast cells in allergic inflammation, which reveals the pathogenesis and the mechanism of an effective therapy for allergies.

Materials and Methods

Mast cells and cross-linking of FcerI

MC9 is an IL-3-dependent murine mast cell line derived from fetal liver cells of a B6 × A/JF1 mouse and kindly provided by Dr. Y. Kitamura (Osaka University, Osaka, Japan). MC9 cells were cultured in RPMI 1640 with 10% FCS, 1 × 10−3 mol/L 2-ME, and antibiotics supplemented with

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2 Abbreviations used in this paper: GCS, glucocorticoids; DXM, dexamethasone; GCR, glucocorticoid receptor; GCRIP, GCR-interacting protein; PI, propidium iodide; SCF, stem cell factor.
CAGGGTCATTGAC-3

and GCRIP1, AACTGTTACCAGCCACTGTG-3

chased from Sigma.

isotype control of rat IgG2a and IgG2b was purchased from Cedarlane.

MC9, cells were incubated with 10 μg/ml of anti-DNP IgE (Sigma, St. Louis, MO) for 12 h at 37°C. Then these cells were washed twice with PBS and resuspended in the above medium without IL-3. Finally, IgE-sensitized cells were added with 500 ng/ml DNP-albumin (Sigma), incubated for the appropriate times, and used for assays.

Cytokines and Abs

Murine rIL-3 was a gift from Dr. Y. Oumoto (Otsukaiseyaku, Tokushima, Japan). A rat anti-mouse IL-3 mAb was purchased from Life Technologies (Gaithersburg, MD). Murine rIL-4 and anti-mouse IL-4 mAb were gifts from Dr. T. Honjo (Kyoto University, Kyoto, Japan). Murine rGM-CSF and a rabbit anti-mouse GM-CSF mAb were gifts from Dr. T. Sudo (Biomaterial Research Institute, Kanagawa, Japan). Murine rL-10 and rSCF (stem cell factor) were purchased from DIALCONE Research (Boulevard Fleming, France). A rat anti-mouse ICAM-1 mAb and a rat anti-mouse LFA-1 mAb were purchased from Seikagaku Kogyo (Tokyo, Japan). The isotype control of rat IgG2a and IgG2b was purchased from Cedarlane (Ontario, Canada). A rabbit IgG and dexamethasone (DXM) were purchased from Sigma.

Measurement of cell viability

Cell viability was measured by a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). Briefly, 5 × 10⁴ cells/well were incubated in 96-well flat-bottom microtiter plates with or without cytokines or other reagents for 48 h. Thereafter, 10 μl/well WST-8 solution was added and incubated for another 4 h, and the absorbance was measured at 450 nm by a Titertek Multiscan Plus ELISA reader (Flow Laboratories, McLean, VA). Each experiment was performed in triplicate, and the results were expressed as A450.

Measurement of apoptosis by flow cytometry

We studied apoptosis by flow cytometry, as described (24). Briefly, cells were washed twice with PBS, and each pellet was dissolved in 1 ml of hypotonic fluorochrome solution (propidium iodide (PI), 50 μg/ml in 0.1% sodium citrate containing 0.1% Triton X-100). These samples were placed in the dark overnight, and the PI fluorescence of individual nuclei was measured using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA). The data were plotted on a logarithmic scale.

RT-PCR analysis

Total RNA was extracted from MC9 cells using guanidium isothiocyanate method. Total RNA (5 μg) was reverse transcribed with MuLV reverse transcriptase. The products obtained by reverse transcription were PCR amplified using sets of primers on a thermal cycler (Atto, Tokyo, Japan). Amplification was done at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. Each cDNA was amplified for 30 cycles. The following sense and antisense primer sets were synthesized with a Model 381A DNA synthesizer (Applied Biosystems, Foster City, CA): IL-3, 5′-GGGAAGAAGCTCTCAGACGCTCTCCTCGTGAAG-3′; SCF, 5′-GGCCAGAAACTAGATCCTTTTGAGTAATGAGCC-3′; IL-4, 5′-TGCCTCATTGTCATCTCTGCT-3′ and 5′-AAGGTGTCCTCGACGTCCTAAG-3′; IL-5, 5′-GAGAAGAAATCTTCCAGGGGTCTC-3′ and 5′-GGCTCACAGCTTTCATGTC-3′; IL-10, 5′-GGGAGGAAGACAAATACGCT-3′ and 5′-CATTTCCGGATGAGAATTGCT-3′; IL-13, 5′-ATGGCCTGCTGGTGACTGACCTGC-3′ and 5′-GAGGAGGCGCGCGGAGACCATGTTG-3′; IL-15, 5′-GGCCATTGTCTTGGTTTCTTCGACGCGAG-3′; GM-CSF, 5′-GCGCTGAACCTCCTGCTCG-3′; and 5′-GCCGGGAAGATCCTCCTCG-3′; TNF-α, 5′-GGGAGGCTTCTTTGTTGGATGTTGAC-3′ and 5′-TATCCTGCTGGCTCACGCTG-3′; SCF, 5′-GGCCACAGACATGACGATCC-3′ and 5′-AAGACCTTACGCGCTGACGACG-3′; IL-11, 5′-AAGGCTTACGCGCTGACGACG-3′ and 5′-ATGACATGCTGACGACGACG-3′; IFN-γ, 5′-TGCACAGACTTGTCGCTGACGACG-3′ and 5′-ACGTAGGAACCTTACGCGCTGACGACG-3′; IL-12, 5′-TGCACAGACTTGTCGCTGACGACG-3′; IL-13, 5′-TGCACAGACTTGTCGCTGACGACG-3′; IL-15, 5′-TGCACAGACTTGTCGCTGACGACG-3′; IL-17, 5′-TGCACAGACTTGTCGCTGACGACG-3′; IL-18, 5′-TGCACAGACTTGTCGCTGACGACG-3′; IL-23, 5′-TGCACAGACTTGTCGCTGACGACG-3′; IL-24, 5′-TGCACAGACTTGTCGCTGACGACG-3′; and IL-25, 5′-TGCACAGACTTGTCGCTGACGACG-3′.

We examined the transcription levels of a number of cytokine mRNA in unstimulated and stimulated MC9 cells via FceRI. Cells were incubated with IgE for 12 h, washed to remove unbound Ab, exposed to specific Ag for 4 h, and harvested for RT-PCR analysis. Unstimulated cells not incubated with IgE and Ag were also analyzed. Total RNA from these cells was reverse transcribed and then PCR amplified using pairs of primers for various cytokines.

mRNA for β-actin was measured to verify the integrity of the total RNA. As shown in Fig. 2, MC9 cells expressed all but two (IL-15 and SCF) of the mRNA tested in response to activation by IgE and Ag, but after activation only low levels of IL-4 and IL-13 were detectable. The expression of IL-3 and GM-CSF mRNA was detectable, but was not significantly enhanced by activation. Taken together with the results of MC9 cell survival by the cross-linking of FceRI or exogenous cytokines, as shown in Fig. 1, these findings indicate the possibility of autocrine cell survival of activated MC9 cells via production of IL-3 or GM-CSF.
with DNP-albumin, a high concentration (50 μg/ml) of anti-IL-3 mAb and/or anti-GM-CSF mAb was added to the IgE-sensitized MC9 cells. After 48 h, we measured the percentages of apoptotic cells by flow cytometer after PI staining. As shown in Fig. 3A, apoptosis was inhibited by the cross-linking of FcεRI on MC9. However, the survival via cross-linking of FcεRI was inhibited by neither anti-IL-3 mAb nor anti-GM-CSF mAb, but inhibited by the combination of both of them. This indicates either IL-3 or GM-CSF is enough for cell survival via cross-linking of FcεRI.

FIGURE 1. The viability of MC9 cells stimulated with exogenous cytokines or cross-linking of FcεRI. A, The MC9 cells were cultured with medium alone, rIL-3 (50 U/ml), rGM-CSF (50 U/ml), or a combination of rIL-3 and rGM-CSF, rIL-4 (50 U/ml), or rSCF (50 U/ml). The MC9 cells were also cultured with anti-DNP IgE (10 μg/ml) and DNP-albumin (500 ng/ml). After 48-h culture, the viability was examined as described in Materials and Methods. Data represent the means ± SD of five replicates. B, Induction of apoptotic cell death in MC9 cells. The MC9 cells were cultured with same dose of cytokines or anti-DNP IgE and DNP-albumin. After 48 h, 1 × 10⁶ cells were stained with PI. Percentages of apoptotic cells were determined using a FACSCalibur flow cytometer. A representative of three experiments is shown.

IL-4 induces self-aggregation of cultured MC9 cells
A total of 10 ml of 1 × 10⁶/ml MC9 cells with IL-3 was maintained in culture dishes. The addition of IL-4 consistently induced the self-aggregation of MC9 cells, as shown in Fig. 4B. Without IL-4, few aggregates were observed (Fig. 4A). Other cytokines tested, including IL-5, IL-10, GM-CSF, TNF-α, and SCF, failed to induce aggregation of MC9 cells (data not shown). Aggregation was observed as early as 8 h after the addition of IL-4, and reached maximum intensity in 3 days. IL-4 could induce aggregation of MC9 cells without IL-3 8 h after the addition of IL-4 (data not shown). However, MC9 cells underwent apoptosis later because of the absence of growth factor. We next examined the effects of several mAbs specific for adhesion molecules on IL-4-induced
self-aggregation of MC9 cells. As shown in Fig. 4E, the aggregation of MC9 cells induced by IL-4 was completely blocked by the addition of mAbs specific for LFA-1α. To identify the IL-4-induced adhesion molecules causing aggregation, we examined the expression of LFA-1α on MC9 cells cultured with and without IL-4 by flow cytometry. In the absence of IL-4, MC9 cells constitutively expressed low levels of LFA-1α. When IL-4 was added to MC9 cells, the expression of LFA-1α markedly increased, as shown in Fig. 4I. These results suggest that IL-4 induces the enhanced expression of LFA-1α, resulting in self-aggregation of MC9 cells. In addition, ICAM-1, which was the high affinity ligand of LFA-1α, was constitutively expressed at a high level on MC9 cells. However, ICAM-1 expression was not enhanced by cross-linking of FcεRI (Fig. 6E). These results indicate that the IL-4-induced aggregation of MC9 cells is mediated mainly by the LFA-1/ICAM-1 interaction.

**LFA-1/ICAM-1 interaction is necessary for the autocrine survival of MC9 cells**

Self-aggregation was also induced via cross-linking of FcεRI on MC9 cells, as shown in Fig. 4C. In addition, self-aggregation induced via cross-linking of FcεRI was completely inhibited by anti-LFA-1α or anti-IL-4 mAb, as shown in Fig. 4, F and I. We next examined the expression of LFA-1 on MC9 cells induced via stimulation of FcεRI. As shown in Fig. 4K, cross-linking of FcεRI induced LFA-1 expression as well as IL-4 on MC9 cells, but it was inhibited by anti-IL-4 mAb, suggesting that the endogenous IL-4 induced the expression of LFA-1 and resulted in aggregation of MC9 cells. To determine whether the LFA-1/ICAM-1 interaction is necessary for the autocrine survival of MC9 cells, we used mAbs specific for LFA-1α and ICAM-1. Before cross-linking of FcεRI with DNP-albumin, high concentration (50 μg/ml) of anti-LFA-1α mAb and/or anti-ICAM-1 mAb or rat isotype control IgG was added to the IgE-sensitized MC9 cells. After 48 h culture, we measured the percentages of apoptotic cells by flow cytometer. As shown in Fig. 5, the survival of MC9 cells by cross-linking of FcεRI was inhibited by either anti-LFA-1α mAb or anti-ICAM-1 mAb. The combination of both of them most efficiently inhibited MC9 cell survival via FcεRI, suggesting the necessity of the LFA-1/ICAM-1 interaction for the autocrine survival of MC9 cells. Taken together, the endogenous IL-4 induced the self-aggregation of MC9 cells, and the local concentration of endogenous IL-3 or GM-CSF is increased by contact cell to cell interaction, by which they exhibit greater effect.
FIGURE 4. Self-aggregation and LFA-1 expression of MC9 cells cultured with cytokines. A total of 10 ml of $1 \times 10^5$/ml MC9 cells was cultured in dishes with A, rIL-3 (50 U/ml); B, rIL-3 (50 U/ml) and rIL-4 (50 U/ml); and C, anti-DNP IgE and DNP-albumin. D–F, A total of 50 μg/ml of anti-LFA-1 α mAb was added to each culture of A–C. G–I, A total of 50 μg/ml of anti-IL-4 mAb was added to each culture of A–C. The cells were incubated for 12 h. This experiment demonstrated similar results three times. J, MC9 cells were cultured with or without rIL-4 (50 U/ml) for 8 h. Then the cells were incubated with either anti-LFA-1 mAb or isotype control rat IgG2a, followed by staining with FITC-conjugated goat F(ab')₂ anti-rat IgG mAb, and then analyzed by a FACSCalibur flow cytometer. K, MC9 cells were cultured in the presence of anti-DNP IgE and DNP-albumin with or without anti-IL-4 mAb (50 μg/ml). Then the cell surface LFA-1 was detected by same method as J.
Glucocorticoid suppresses autocrine survival of MC9 cells by inhibiting endogenous IL-4 production and ICAM-1 expression

Since GCS are widely used to control the symptoms of allergic patients, it was important to examine whether GCS shows a direct effect on mast cells. We first studied the mRNA expression of glucocorticoid receptor (GCR) in unstimulated MC9 cells by RT-PCR. As shown in Fig. 6A, MC9 cells constitutively expressed the message for GCR and GCR-interacting protein 1 (GCRIP1). We next examined the effects of DXM on the survival of MC9 cells stimulated with cytokines or cross-linking of FcεRI. DXM (1 μM) was added to MC9 cell cultures 4 h before stimulation with cytokines or cross-linking of FcεRI. After 48-h culture, we evaluated the survival of MC9 cells. As shown in Fig. 6B, DXM did not affect the cell survival stimulated with exogenous IL-3 or GM-CSF, but markedly inhibited the survival via cross-linking of FcεRI. To define the mechanism by which DXM inhibited the survival of MC9 cells stimulated via FcεRI, we studied the effect of DXM on the level of mRNA for cytokines such as IL-3, IL-4, and GM-CSF. DXM (1 μM) was added to MC9 cell cultures 4 or 48 h before stimulation via cross-linking of FcεRI. After 4-h culture, we extracted the mRNA and evaluated the mRNA level of cytokines by Northern blot analysis. As shown in Fig. 6C, DXM significantly inhibited the FcεRI-stimulated increase in the message for IL-4. In contrast, DXM did not affect the FcεRI-stimulated increment in IL-3 or GM-CSF message. In addition, as shown in Fig. 6D, the inhibition of FcεRI-mediated survival by DXM was overcome by the addition of IL-4, suggesting that DXM inhibited the induction of IL-4 via cross-linking of FcεRI. Finally, we determined the effect of DXM on expression of ICAM-1 and LFA-1 on MC9 cells. MC9 cells were cultured with DXM (1 μM) for 8 h in the presence of IL-3 or stimulation of FcεRI, and the expression of ICAM-1 and LFA-1 was measured by flow cytometry. As shown in Fig. 6E, DXM markedly diminished both constitutive expression of ICAM-1 and FcεRI-stimulated increase of LFA-1 expression on MC9 cells, suggesting the inhibition of LFA-1/ICAM-1 interaction of MC9 cells by DXM.

Discussion

Mast cells produce multiple cytokines upon stimulation via cross-linking of FcεRI. In this study, we demonstrated that mast cells accumulate mRNA of multiple cytokines such as IL-3, IL-4, IL-5, IL-10, IL-13, GM-CSF, and TNF-α after stimulation through the IgE receptor. On the other hand, cytokines that have growth activity on mast cells were also reported, such as IL-3 and SCF (25). IL-3 is necessary for the growth of mucosal-type mast cells, while SCF is required for the maintenance of connective tissue-type mast cells (26). Recently, a novel IL-15R was identified on mast cells (27). These findings support the possibility of autocrine growth of mast cells activated via cross-linking of FcεRI. In this study, we demonstrated the autocrine survival of IL-3-dependent MC9 cells via production of IL-3 and GM-CSF, but SCF and IL-15 were not produced after stimulation through the IgE receptor. In addition, we demonstrated not only IL-3 and GM-CSF, but also IL-4 production is necessary for MC9 cell survival induced via cross-linking of FcεRI. The synergistic effects of cytokines on mast cells have been reported. For example, IL-4 enhances the proliferation of mast cells by IL-3 (9), and IL-10 also enhances the IL-3- and IL-4-dependent growth of mast cells (28). But these cytokines were added at exogenously excess amounts for the growth of mast cells in vitro, so the possibility remains that local conditions such as the inflammatory site in vivo were not reflected in these previous studies. Moreover, the mechanisms of synergistic effects of these cytokines were not defined. In this study, we identified the mechanism of autocrine survival of mast cells and the synergistic effects of endogenous cytokines produced after stimulation by cross-linking of FcεRI. It mimics more closely in vivo conditions. These findings support the idea that IL-3 represents the major cytokine regulating the survival and proliferation of mucosal mast cells (29), while IL-4 is required to maintain cell to cell contact so that the local concentration of endogenous IL-3 and GM-CSF is increased and they can work effectively at inflammatory sites.

Thus, IL-4 is a multifunctional cytokine that appears to play an important role in the pathogenesis of allergic diseases. Clinically, in atopic patients, increased numbers of cells positive for IL-4 mRNA are demonstrated during the allergen-induced late-phase cutaneous response, suggesting IL-4 is up-regulated in human allergic disease (10). Dysregulation of IL-4 gene expression was also reported in steroid-resistant asthma (13). Abs to IL-3 and IL-4 suppress helminth-induced intestinal mastocytosis (14). Allergen immunotherapy decreases IL-4 production in CD4+ T cells from allergic individuals (11, 12). Although the mechanisms of IL-4 in allergic inflammation are not clearly defined, the enhanced expression of several adhesion molecules by IL-4 is reported. IL-4 induces LFA-1 and LFA-3 expression on Burkitt’s lymphoma cell line (30). ICAM-1 expression is regulated by IL-4 in human dermal fibroblasts (31) and human monocytic tumor cell lines (32). On the other hand, mast cells also express several adhesion molecules (33, 34). Expression of integrins differs in murine mast cells cultured with different cytokines (35, 36). ICAM-1 expression is regulated by IL-4 in human mast cell line (37). IL-3-induced DNA synthesis and proliferation of mast cell are augmented by integrin-mediated adherence of the cells to extracellular matrix (38). Thus, cytokines and adhesion molecules work synergistically in the inflammatory sites. IL-4 and TNF-α also induce adhesion molecules on endothelium, which plays an important role in infiltration of inflammatory cells to tissues (39). Indeed, activation of mast cell enhances this migration (40). In addition, IL-4 induces aggregation of human mast cells by promoting LFA-1/ICAM-1 (41), as shown in this study. Moreover, IL-4 induces a class switch of Ig to IgE in B cells. Thus, the mechanisms by which IL-4 and adhesion molecules play a critical role in allergic pathogenesis have been gradually defined. In this study, we suggest one possible model for the local role of IL-4 and adhesion molecules on mast cells at inflammatory sites in allergic diseases.
FIGURE 6. GCR expression and the inhibition of MC9 cell viability induced by cross-linking of FcεRI with DXM. A, Total RNA (5 μg) from MC9 cells was reverse transcribed and PCR amplified, as described. Products using primers for GCR and GCRIP1 were resolved in 2% agarose gels, visualized by staining with ethidium bromide, and analyzed by a FMBIO-100 image analyzer. B, MC9 cells were cultured in the presence of medium alone, rIL-3 (50 U/ml), rGM-CSF (50 U/ml), a combination of rIL-3 and rGM-CSF, or anti-DNP IgE and DNP-albumin with or without DXM (1 μM). After 48-h culture, the viability was examined, as described in Materials and Methods. Data represent the means ± SD of five replicates. C, MC9 cells were cultured in the presence of IL-3 with DXM (1 μM) for 4 or 48 h before stimulated with anti-DNP IgE and DNP-albumin. After 4-h stimulation, the mRNA was extracted, blotted onto nylon membrane, and hybridized with labeled cDNA probes for indicated cytokines and β-actin. D, The MC9 cells were cultured in the presence of anti-DNP IgE and DNP-albumin with DXM (1 μM) alone or DXM and rIL-4 (50 U/ml). After 48-h culture, the percentages of cell viability were determined using a flow cytometer after PI staining. E, MC9 cells were cultured in the presence of IL-3 or anti-DNP IgE and DNP-albumin with or without DXM (1 μM) for 8 h. Then the cells were incubated with anti-ICAM-1 mAb, isotype control rat IgG2a, or anti-LFA-1α mAb, followed by staining with FITC-conjugated goat F(ab‘)2 anti-rat IgG mAb, and analyzed by a FACSCalibur flow cytometer.
GCS are known as potent antiinflammatory and immunosuppressive agents with a more profound effect on cell-mediated immune response than on Ab production. The mechanisms by which GCS suppress the immune response are complex because GCR exist in various cell types involved in immune responses, and the molecules regulated by GCR are different in each cell. The effects of GCS are most minutely examined in lymphocytes. GCS induce apoptosis in immature and activated T cells (42). GCS also suppress IL-2 production in T cells stimulated with TCR, resulting in the suppression of T cell proliferation (43, 44). GCS suppress the production of several cytokines by activated T cells, such as IL-3, IL-4, IL-5, IL-6, IL-8, GM-CSF, and TNF-α (45–47). Not only do GCS strongly suppress the T cell function, but few suppressive effects on B cells have been reported. Recently, the direct effects of GCS on mast cells were reported. IgE-mediated mediator release from mast cell was regulated by GCS (48). In addition, GCS also inhibit the c-kit ligand- and IL-3-induced proliferation of bone marrow-derived mast cell (49). However, no proliferation measured by thymidine incorporation does not necessarily mean cell death. Moreover, DXM does not directly induce death of mast cells (50). Although the different results were reported in some systems, our results obviously indicated that DXM did not induce direct cell death of MC9 cells. On the other hand, it was also reported that cytokine production such as TNF-α via cross-linking of FceRI was inhibited by GCS (49, 51, 52). The mechanisms by which TNF-α production were inhibited by GCS were different among these studies. One of them reported the possibility of post-transcriptional regulation by GCS (52). However, our results about IL-4 production by Northern blot indicated the inhibition at the transcriptional level. In addition, components of a mast cell-spe-cific IL-4 intronic enhancer were reported (53). These findings indicate the possibility that cytokine expression in mast cells is specifically regulated by own mechanisms, including the effects of GCS. In this study, we demonstrated the direct effect of GCS on mast cells and revealed the suppression of mRNA expression for IL-4, but not for IL-3 and GM-CSF, confirming the different effects of GCS on different cells involved in immune responses. GCS suppress the expression of adhesion molecules on endothelium such as ELAM-1 and ICAM-1 (54), resulting in the suppres-sion of infiltration of inflammatory cells. We also defined the de-creased expression of ICAM-1, which is constitutively expressed on MC9 cells, confirming the above effects of GCS.

Mast-cell-derived cytokines induce not only autocrine cell growth, but also expression of adhesion molecules on endothelium, which results in the adhesion of lymphocytes and eosinophils to endothelium and infiltration of these cells at inflammatory sites. In fact, the synthesis and secretion of IL-4 and IL-5 by mucosal mast cells are seen in allergic rhinitis and asthma patients by immunohistochemistry or in situ hybridization (55, 56). Thus, mast cells play a critical role in the production of cytokines, infiltration of inflammatory cells, and the induction of late response of allergy. In addition, the survival and proliferation of mast cells at inflammatory sites result in the prolongation of allergic inflammation. Autocrine cell survival and proliferation are known in T cells via production of IL-2 upon stimulation of TCR, or bone marrow-derived cells via production of IL-3, stimulated through FcγRIII (57). Under physiologic conditions, the proliferation and survival of these cells are transient and followed by activation-induced cell death through the Fas/Fas ligand system (57, 58). In this study, we observed the expression of neither Fas nor Fas ligand after activa-tion of MC9 cells via cross-linking of FceRI (data not shown). Therefore, the apoptosis induced by withdrawal of IL-3 is probably important for the regulation of mast cells. In this aspect, it was reported that GCS inhibited IL-3 expression of human colo-rectal FcεRII-positive cells in patients with inflammatory bowel dis-ease (59). GCS also decreased the number of tissue mast cells by reducing the production of SCF by fibroblasts (60). In this study, we demonstrate that GCS directly inhibits increased mRNA for IL-4 in MC9 cells stimulated via cross-linking of FceRII accompanied with the decrease of ICAM-1 expression. Inhibited IL-4 production induced the decrease of LFA-1 expression and self-aggregation through LFA-1/ICAM-1 interaction, resulting in the induction of apoptosis of mast cells by diminishing the relative low concentrations of endogenous IL-3 and GM-CSF. These findings provide a model for the local interaction of mast cells, which could play an important role in allergies and the mechanisms by which GCS control the pathogenesis of allergic inflammation.

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