Glucocorticoids Decrease Tissue Mast Cell Number by Reducing the Production of the c-kit Ligand, Stem Cell Factor, by Resident Cells

In Vitro and In Vivo Evidence in Murine Systems

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Abstract

The local delivery of glucocorticoids to tissues significantly decreases mast cell number. This pharmacologic effect of glucocorticoids is believed to be one of the mechanisms by which glucocorticoids regulate allergic inflammation. To determine the mechanism by which glucocorticoids are able to exert this effect, we first applied the glucocorticoid fluocinonide to mouse dermis and observed that the decrease in mast cell number was associated with an increase in mast cell apoptosis. This did not appear to be due to a direct effect of the glucocorticoid on mast cells, as the addition of 0.01–1.0 μM of the glucocorticoid dexamethasone into stem cell factor (SCF)-dependent mast cell cultures did not enhance mast cell death. However, addition of dexamethasone to cultured fibroblasts did result in a downregulation of SCF mRNA and a significant decrease in SCF protein production. Similarly, immunohistochemistry performed on fluocinonide-treated mouse dermis revealed a decrease in immunoreactive SCF. Administration of SCF at sites of fluocinonide administration to the dermis abolished the mast cell–depleting effect of this glucocorticoid. Thus, glucocorticoids decrease tissue mast cell number by downregulating tissue SCF production required for the survival of local mast cells. This observation may be applicable to the design of improved strategies to treat mast cell–mediated disorders. (*J. Clin. Invest.* 1997. 99:1721–1728.) Key words: glucocorticoids • stem cell factor • apoptosis • mast cells • fibroblasts

Introduction

Mast cells resident in tissues are capable of inducing or potentiating local inflammation after activation by a variety of stimuli including interactions between antigen and membrane-bound IgE or IgG; and exposure to C3a or C5a (1–7). Glucocorticoids delivered at potential or on-going sites of tissue inflammation decrease the number of resident tissue mast cells, an effect that contributes to the antiinflammatory properties of these agents. Thus, glucocorticoids applied to the skin (8, 9), lung (10), and intestine (11) result in a significant reduction in mast cells. However, the mechanism by which glucocorticoids reduce tissue mast cell number is not known. Understanding the way in which glucocorticoids decrease mast cell number could lead to improved strategies to interrupt mast cell–mediated inflammation while limiting the side effects of locally administered steroids.

The number of tissue mast cells is believed to be regulated in the microenvironment principally by the c-kit ligand, stem cell factor (SCF)[1] (12–14) which is produced by resident cells such as fibroblasts (15–18). Withdrawal of SCF from SCF-dependent mast cell cultures in vitro results in apoptosis (19, 20), and administration of SCF in vivo in both murine and human studies leads to both local and systemic mast cell hyperplasia (21–23). Therefore, we hypothesized that glucocorticoids affect mast cell number in tissues by regulating SCF production by cells in the microenvironment.

To explore this hypothesis, we performed a variety of in vitro and in vivo experiments. As will be shown, glucocorticoids do not appear to directly alter mast cell number by acting on mast cells themselves. Rather, glucocorticoids decrease the production of SCF by fibroblasts in vitro and resident connective tissue cells in vivo; and the mast cell–depleting effects of glucocorticoids in vivo are reversed by the local administration of SCF.

Methods

Materials. DME, glutamine, penicillin, and streptomycin (Gibco Laboratories, Grand Island, NY); FBS (Bioproducts for Science, Inc., Indianapolis, IN); water-soluble dexamethasone (Sigma Chemical Co., St. Louis, MO); Lidex (fluocinonide) ointment 0.05% (Syntex Laboratories, Inc., Palo Alto, CA); recombinant murine SCF (rm-SCF) expressed in *Escherichia coli* (R & D Systems, Minneapolis, MN); polyclonal rabbit anti–mouse SCF (Genzyme Corp., Cambridge, MA); normal goat serum (BioSource, International, Camarillo, CA); normal rabbit serum (Dako Corp., Carpinteria, CA); Vectastain ABC-AP kit and alkaline phosphatase substrate kit (Vector Labs, Inc., Burlingame, CA); Levamisole (Sigma Chemical Co.); biotinylated anti–rabbit IgG (Vector Labs, Inc.); Cy3™-conjugated streptavidin (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA); and ApoTag™ Plus in situ apoptosis detection kit (Oncor Inc., Gaithersburg, MD) were obtained from the manufacturers.

Cell cultures. Human fetal skin and NIH 3T3 fibroblasts (passage 5, CRL 1475-CCD27SK) were purchased from American Type Culture Collection (Rockville, MD). The cell cultures were maintained in DME with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 50 μg/ml streptomycin. MCP5 mast cells of Balb/c origin (19) were maintained in RPMI 1640 with 10% heat-inactivated FBS, 50 mM

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1. *Abbreviations used in this paper:* PI, propidium iodide, RPA, ribonuclease protection assay; SCF, stem cell factor.

β-mercaptoethanol, 2 mM L-glutamine, 25 mM Hepes, 1 mM sodium pyruvate, nonessential amino acids, 100 U/ml penicillin, 50 mg/ml streptomycin, and 10% (vol/vol) WEHI-3 conditioned medium as a source of IL-3. rmSCF was added (70 ng/ml) to some of the cell suspensions where indicated.

RNA samples and reverse transcription. Human fetal skin fibroblasts in subconfluent cultures were washed with PBS and incubated three times for 24 h with or without specified amounts of dexamethasone. At the end of each experiment, adherent fibroblasts were detached with trypsin/versene (Biofluids, Inc., Rockville, MD), counted, washed twice in PBS, lysed in 4 M guanidinium isothiocyanate and total RNA isolated using guanidine thiocyanate/phenol-chloroform extraction as described (24).

cDNAs were synthesized from total RNA with avian myeloblast virus reverse transcriptase and random hexamer primers (cDNA Cycle™ kit for RT-PCR; Invitrogen Corp., San Diego, CA). Two oligonucleotides were synthesized (Operon Technologies, Inc., Alameda, CA) that correspond to the sequence in cDNA for human (h)SCF (oligonucleotide I: 5′-CTTCAACATTAAGTCCTGAG-3′ and oligonucleotide II: 5′-CAGTGTTGATACAAGCCACA-3′). These oligonucleotides amplify a 359-bp and a 275-bp product of SCF mRNA when exon 6 is present or deleted by alternative splicing, respectively (25, 26). cDNA amplification was performed at 40 cycles each of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min.

Probe construction and labeling. The 359-bp PCR product of SCF mRNA obtained as described above was cloned in a pBluescript II SK(+/−) vector at the EcoRV site of the polylinker using blunt end ligation. The new construct was then sequenced according to the dideoxy-chain-termination method with a Sequenase kit, version 2.0 (United States Biochemical Corp., Cleveland, OH). The plasmid was then linearized by restriction digestion with XhOI. The linearized DNA template was in vitro transcribed with the MAXIscript™ system (Ambion Inc., Austin, TX) using T3 RNA polymerase to obtain an antisense probe for detecting SCF mRNAs. In vitro transcribed antisense RNA was uniformly labeled with [α-^{32}P]uridine triphosphate (Amersham, Arlington Heights, IL). After in vitro transcription, the DNA template was digested with RNase-free DNase I. The probe was then purified with a 7 mol/liter urea/6% polyacrylamide gel (National Diagnostics, Inc., Atlanta, GA). The full-length probe (483 nucleotides [nt]) was subsequently used in a ribonuclease protection assay (RPA). Hybridization of this transcript to human total RNA protects a 359 nt fragment of SCF which extends from nucleotide 679 to 1038 of the cDNA sequence (GenBank accession No. MC59964) (26). The pTRI-β-actin-human antisense control template contains a 245-bp fragment of the human cytoplasmic β-actin gene which extends from codon 220 to 303 (nucleotides 704–947 of cDNA sequence, GenBank accession No. X00351). The β-actin fragment was inserted into the kpnI-EcoRI sites of a pTRIPLEscript™ vector (Ambion Inc.). The plasmid was linearized with XbaI and HindIII, and the linearized DNA template was in vitro transcribed as above using T7 RNA polymerase, gel purified to obtain a fragment of 304 nt, and then used in the RPAs. Hybridization of the transcript to human total RNA protects a 245 nt fragment of β-actin mRNA.

RPA. Preliminary studies demonstrated that SCF mRNA levels in fibroblasts were at the lower limits of detection of standard Northern blots, therefore the RPA was utilized employing a commercially available kit (RPA II; Ambion Inc.). Each 10 μg of total RNA was hybridized overnight at 43°C to 50,000 cpm of gel purified antisense probe, followed by RNase T1 (100 U/ml) digestion for 30 min at 37°C. RNase digestion was terminated by inactivation of the enzyme followed by ethanol precipitation. Digestion products were subjected to gel electrophoresis using a 7 mol/liter urea/6% polyacrylamide gel (Sequagel; National Diagnostics, Inc.). As control, yeast RNA was hybridized to each probe and then treated with or without RNase T1. In addition, for each experiment the DNA molecular weight marker was labeled with [α-^{32}P]uridine triphosphate and subjected to electrophoresis along with the other samples. Next, Kodak x-ray film (Eastman Kodak, Rochester, NY) was exposed to dried gel at −70°C with intensifying screens. Densitometric analysis was performed using a Molecular Dynamics Scanner (Molecular Dynamics, Sunnyvale, CA). Two separate experiments in duplicate were performed.

SCF ELISA. Fibroblasts were cultured in DME with 10% FBS with and without dexamethasone at 1.00, 0.100, and 0.010 μM for 72 h with medium and dexamethasone replaced at 24 and 48 h. Supernatants corresponding to the last 24 h were collected, concentrated by ultracentrifugation through an anisotrope membrane (Centricon 10; Amicon, Inc., Beverly, MA), and stored at −80°C until analyzed by ELISA. Fibroblasts were then detached with trypsin/versene solution and viability was assessed by trypan blue dye exclusion. Soluble SCF was measured in the concentrated supernatants using a Quantikine™ ELISA kit (R & D Systems). Standards for soluble SCF were prepared using culture medium as diluent. Under these experimental conditions, the minimum detectable dose of SCF is 3.0 pg/ml. SCF levels were corrected to cell number and expressed as pg of SCF/10^6 cells.

SCF detection in 3T3 fibroblasts by immunohistochemistry. 3T3 fibroblasts were cultured on 12-mm-diameter coverslips in 24-well plates in duplicate in DME with 10% FBS with and without 1 μM −20°C. dexamethasone for 72 h with medium and dexamethasone replaced at 24 and 48 h. At the end of the culture, wells were washed in PBS, coverslips were removed from the wells, air dried, and fixed in methanol for 5 min at −20°C. Coverslips were rehydrated in three washes of PBS and incubated for 1 h with 100% heat-inactivated normal goat serum to prevent nonspecific binding. Coverslips were then incubated with rabbit anti–mouse SCF (10 μg/ml) in PBS with 10% normal goat serum overnight at 4°C. The control for nonspecific binding was performed using nonimmune rabbit serum at an equivalent concentration, instead of the primary antibody. The coverslips were washed in PBS, and specific binding was detected with a biotinylated anti–rabbit IgG antibody (1:100) for 1 h at room temperature followed by incubation with Cy3™-streptavidin conjugated antibody (1:500) for 1 h at room temperature. After three washes in PBS, coverslips were rinsed in distilled water and mounted with an aqueous mounting medium. Three experiments in duplicate were performed.

FACS® analysis. At time 0 and at specified time points, cells were analyzed by flow cytometric analysis and by using propidium iodide (PI) (Sigma Chemical Co.) uptake to assess cell viability after growth factor deprivation and dexamethasone treatment. Dexamethasone was added at the beginning of the cultures at the indicated concentrations to cells placed in 96-well plates at 7×10^4 cells/ml. Cells were then collected and suspended at 10^6 cells/ml in PBS containing 0.1% BSA and PI added at a final concentration of 2.5 μg/ml between 5 and 15 min before analysis (FACS 440; Becton Dickinson, Mountain View, CA).

In vivo studies. Groups of three 5–6-mo-old female Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were lightly anesthetized by Methophane inhalation before injection. Intradermal injection of an ear was performed three times a week for 2 wk with vehicle alone (sterile saline with 0.1% BSA, fraction V; Sigma Chemical Co.) or vehicle containing rmSCF (30 μg rmSCF/Kg in 20 μl of sterile saline containing 0.1% BSA). A third group received concomitant daily treatment with fluocinonide (0.05 mg/treatment) at the site of rmSCF injection. An additional control group was treated with fluocinonide alone. In additional experiments, the left ear was treated with fluocinonide and the right ear was not treated or treated with an ointment control cream (Aquaphor®; Beiersdorf Inc., Norwalk, CT). Mast cell number per mm^2 was determined as described (27) with modifications. Briefly, both ears from each of three mice per condition were removed after death by cervical dislocation, fixed in 10% neutral buffered formalin for 24 h, immersed in 70% ethanol, and embedded in paraffin. Three tissue sections (4 μm) from each ear, unless otherwise stated, were placed on polylysine-coated slides and stained with Lennert's Giemsa at pH 0.5 (28). Each section was examined at 400× and dermal mast cells were randomly counted in 15 fields. The average number of mast cells in 15 fields was determined and the mean

number of mast cells per mm^2 in each ear was calculated by averaging the results obtained from each section. This final determination was considered as one observation. Mast cells as percentage of control was determined by comparing mast cell number under each condition and time point to the mean number of mast cells per mm^2 in control ears.

Immunohistochemistry. For immunostaining, 4-μm tissue sections were floated onto polylysine-coated slides, dewaxed through xylene, rehydrated through an ethanol series, washed in PBS, and incubated in 100% heat-inactivated normal goat serum to prevent nonspecific binding. The slides were then subjected to staining for SCF protein using rabbit anti–mouse SCF (10 μg/ml) in PBS with 0.1% BSA and 10% normal goat serum overnight at 4°C. The control for nonspecific binding was performed by omitting the primary antibody and by using nonimmune rabbit serum at an equivalent concentration instead of the primary antibody. The sections were washed with PBS. Specific binding was detected by the Vectastain ABC-AP and alkaline phosphatase substrate kits according to the manufacturer's instructions. Endogenous alkaline phosphatase was blocked using 1 μM Levamisole in the substrate solution. Slides were counterstained with hematoxylin solution, Gill No. 2 (Sigma Chemical Co.), dehydrated, and mounted in Permount (Fisher Scientific, Pittsburgh, PA). Three mice for each group and six sections for each sample were stained and subsequently analyzed using a light microscope at a magnification of 400.

In situ apoptosis (TUNEL assay). For detection of apoptotic mast cells, ear samples were obtained at specified time points, fixed in 10% buffered formalin at 4°C, embedded in paraffin, and 4-μm sections were mounted on polylysine-coated slides. Next, slides were deparaffinized in xylene, hydrated through an ethanol series, washed in PBS, and subjected to proteinase treatment (20 μg/ml) (Proteinase K; GIBCO BRL, Gaithersburg, MD) for 15 min at room temperature. Endogenous peroxidase was inhibited by 2.5% hydrogen peroxide treatment. Incubation with a digoxigenin-nucleotide triphosphate targets catalytically the 3′-OH ends of fragmented DNA through the enzyme terminal deoxynucleotidyl transferase. As a negative control, water was added instead of terminal deoxynucleotidyl transferase enzyme to the reaction buffer. This treatment was followed by incubation with antidigoxigenin antibody peroxidase conjugate and detection was performed with a peroxidase substrate in accordance with the manufacturer's instructions (ApopTag™ in situ apoptosis detection kit). Slides were briefly rinsed in acid water (pH 0.4) and stained with alcian blue, at pH 0.5, to visualize mast cells. Next, samples were dehydrated through an ethanol series followed by a xylene wash and then mounted in Permount. Three mice per group and 200 alcian blue positive mast cells per mouse were analyzed for each sample at 1,000×. Results are reported as percent apoptotic mast cells.

Results

Depletion of tissue mast cells. It has been reported that corticosteroids applied to the skin result in a decrease in mast cell number (8, 9). To examine this phenomenon, we first applied the glucocorticoid fluocinonide topically to mouse skin and determined the number of cutaneous mast cells over 14 d. As can be seen in Figs. 1 *A* and 2, *A* and *C*, the number of mast cells in the cutaneous tissues of mouse ears decreased by ~ 35% by day 3 and 56% by day 14 ($P < 0.05$, compared with day 0), similar to published reports (8, 9).

To further examine this mast cell depletion, we investigated whether the decrease in mast cell number was accompanied by an increase in the number of mast cells undergoing apoptosis. In fact, fluocinonide treatment was accompanied by an increase in the number of mast cells undergoing apoptosis (Figs. 1 *B* and 2, *B* and *D*). The number of mast cells undergoing apoptosis was maximum at day 3 (7.62±2.7). An increase in mast cells undergoing apoptosis above control continued to be observed through day 14 of the study and was statistically significant ($P < 0.05$) through day 10.

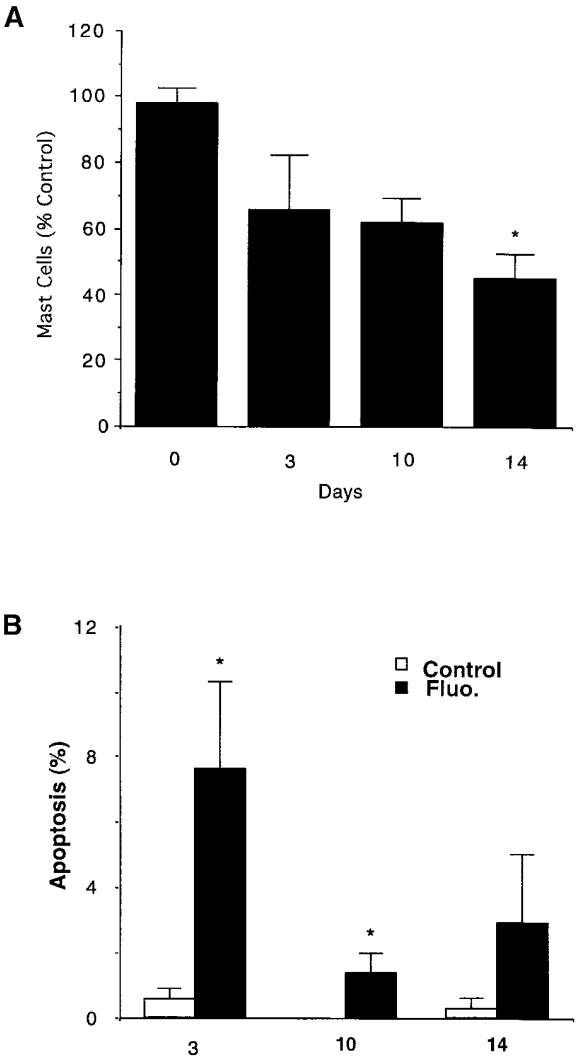


Figure 1. Effect of fluocinonide on mast cell number and number of mast cells in apoptosis in mouse ear dermis. Fluocinonide-induced decrease in mast cell number as percent control is shown in *A*, and the percentage of mast cells undergoing apoptosis over 14 d is shown in *B*. Mast cell numbers were determined as described in Methods. The average number of mast cells in untreated mouse ears was 138±8.7 per mm^2. Data are presented as mean±SEM ($n = 3$). ANOVA and Student's *t* test (two-tailed) were used to compare groups in *A* and *B*, respectively, and where $*P < 0.05$.

Effect of glucocorticoid on mast cell survival in vitro. The observation that the decrease of mast cell number in vivo is accompanied by mast cell apoptosis suggested that glucocorticoids could either act by decreasing the production of SCF by tissue stromal cells which is required to maintain mast cell viability, or alternatively by blocking the ability of SCF to maintain mast cell viability by acting directly on mast cells. To examine the latter possibility, we investigated the ability of dexamethasone to decrease the survival of mast cells in vitro in the presence of SCF (19). As can be seen in Fig. 3 and Table I, mast cells cultured in IL-3 remained viable while mast cells cultured without IL-3 exhibited apoptosis (compare Fig. 3, *A*

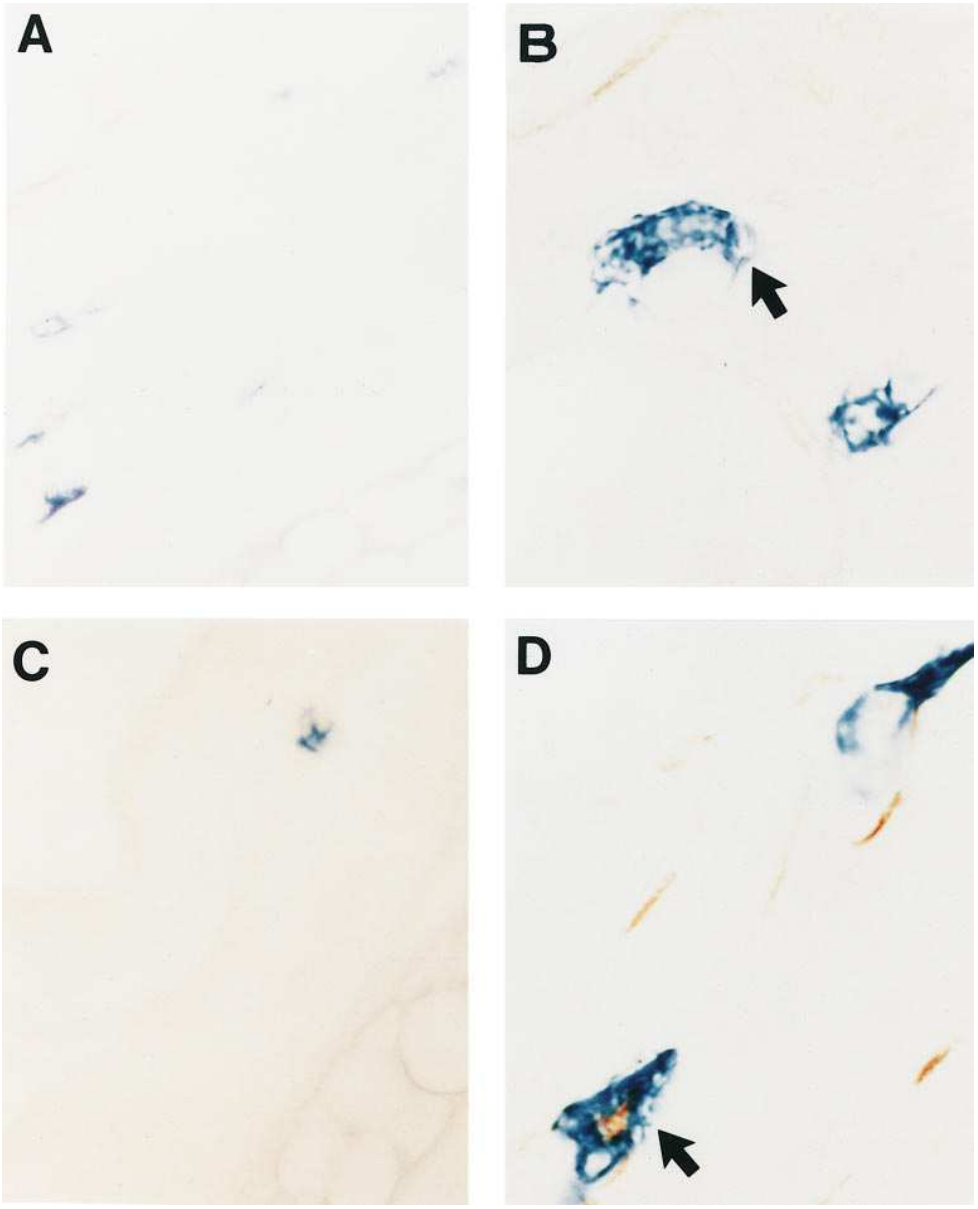


Figure 2. Light microscopy showing mast cells in control and fluocinonide-treated mouse ear dermis. Alcian blue–stained mast cells are easily seen in an untreated section of dermis (×400) (*A*), while mast cells in fluocinonide-treated dermis are decreased in number (*C*). A normal mast cell (*arrow*) at ×1,000 (*B*) and an apoptotic mast cell (*arrow*) at ×1,000 (*D*) differ in that the apoptotic mast cell shows a brown nucleus.

with *B*) as evident by increased PI uptake (19). IL-3–dependent mast cells removed from IL-3 and placed in SCF showed a decrease in PI uptake, although this decrease was less than that observed in growth factor–deprived mast cells (compare Fig. 3, *C* and *A*). The addition of dexamethasone from 0.01 to 1.00 μM did not alter the ability of SCF (or IL-3) to maintain mast cell viability through 96 h (Fig. 3, *D–I*) (Table I). Thus, dexamethasone did not block the effect of SCF in promoting mast cell viability.

Effect of dexamethasone on SCF production by fibroblasts. To examine whether glucocorticoids decrease mast cell number in tissues by downregulating the production of SCF by stromal cells, we first investigated the in vitro effect of dexamethasone on the production of SCF mRNA by human fetal skin fibroblasts. As shown in Fig. 4, when human fetal skin fibroblasts are cultured in dexamethasone (0.1 and 1.0 μM) the amount of SCF mRNA is decreased as assessed by RPA (Fig. 4, *A* and *B*) in a dose–response manner (Fig. 4 *C*).

To verify that the decrease in SCF mRNA is accompanied by a decrease in SCF protein, we added increasing amounts of dexamethasone to human fetal fibroblasts in culture. This resulted in a significant decrease in the amount of SCF protein in the culture supernatants, again in a dose–response manner (Fig. 5 *A*). Thus, 1 μM dexamethasone decreased SCF protein production from 311.0±50.0 to 146.4±21.3 pg 10^6 cells. To rule out the possibility of a cytotoxic effect on human fetal skin fibroblasts from dexamethasone, we measured cell viability by trypan blue dye exclusion at the end of each treatment. No change in fibroblast viability was observed under all experimental conditions (98.75±1.03% and 98.96±1.03% after no treatment or 1.0 μM dexamethasone, respectively; $n = 3$ in triplicate).

Furthermore, to establish that glucocorticoids can decrease SCF in mouse NIH 3T3 fibroblasts as well as in human fibroblasts, we cultured 3T3/NIH mouse fibroblasts on cover slips for 72 h with and without 1 μM dexamethasone. This resulted

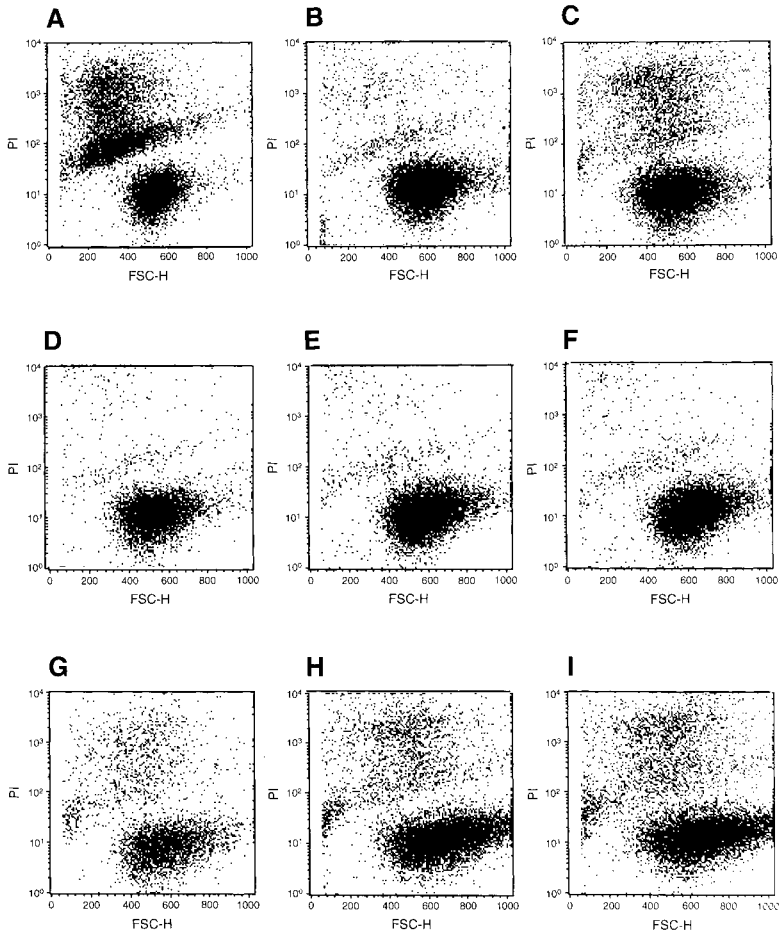


Figure 3. Analysis of PI uptake by flow cytometry in cultured mast cells. IL-3–dependent MCP5 cells were incubated for 48 h without IL-3 or SCF (*A*) or in media containing IL-3 (*B*, *D*, *E*, *F*) or SCF (70 ng/ml) (*C*, *G*, *H*, *I*), containing 0.01 μM (*D* and *G*), 0.1 μM (*E* and *H*), and 1.00 μM (*F* and *I*) dexamethasone before analysis. For details, see Methods.

in a visible decrease in cell-associated SCF after dexamethasone treatment (Fig. 5, *B* and *C*).

In vivo treatment with fluocinonide decreases SCF protein at the treated sites. To confirm the in vitro observation in vivo that glucocorticoids downregulate SCF production by fibroblasts, we next examined normal and fluocinonide-treated mouse skin for the presence of SCF protein using immunohistochemistry. SCF protein was detected in untreated mouse skin within stromal cells and in the epidermis (Fig. 6 *A*) as has been reported in human skin (25). The amount of protein associated with stromal cells appeared to decrease after application of fluocinonide (Fig. 6 *B*). Thus, glucocorticoids decrease SCF production by fibroblasts in vitro and similarly by stromal cells in vivo.

Table I. Analysis of Mast Cell Survival (%) with Dexamethasone (DEX) Treatment

	DEX		
Growth factor (h)	0.01 μM	0.10 μM	1.00 μM
IL-3 (48)	101.28±0.35	101.01±0.68	100.06±0.88
(72)	105.63±2.22	103.62±0.66	103.94±0.48
(96)	106.09±3.33	104.30±2.33	104.65±2.15
SCF (48)	99.32±3.42	101.34±1.22	99.61±1.69
(72)	99.14±2.15	104.58±4.65	104.39±2.14
(96)	96.77±4.82	94.81±3.66	93.44±6.79

The percent cell survival was calculated in 10,000 events by gating the cell population that had low PI uptake. The percent cell survival after dexamethasone treatment was then compared with the percent cell survival without dexamethasone and with either IL-3 or SCF (control). Results are reported as percent cell survival compared with control cultures. Data are presented as mean (±SEM) ($n = 4$).

SCF injection in vivo. The above data are consistent with the hypothesis that application of glucocorticoids to the dermis depletes mast cells by decreasing the amount of SCF produced by stromal cells, and this in turn leads to mast cell apoptosis. If this hypothesis is correct, it should be possible to reverse the effect of fluocinonide by the introduction of SCF at the site of application of the glucocorticoid. As can be seen in Fig. 7, introduction of SCF into mouse skin in the absence of fluocinonide increases mast cell number as reported (20); and as previously noted, application of fluocinonide decreases mast cell number. This depletion of mast cells induced by fluocinonide was reversed with local introduction of SCF ($P < 0.01$), consistent with the hypothesis that the mast cell–depleting effect of glucocorticoids is the result of a downregulation of local SCF production.

Discussion

Local contact of glucocorticoids with dermal, respiratory, and intestinal tissues is reported to be associated with a decrease in

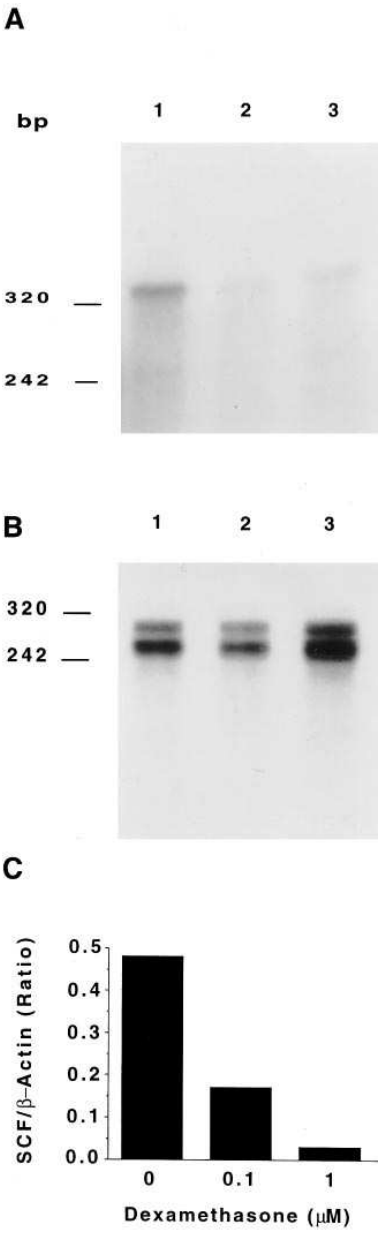


Figure 4. Effect of dexamethasone on SCF mRNA levels in fibroblasts. Autoradiogram of electrophoretic analysis of RNase protection products for SCF (*A*) and β-actin (*B*) from fibroblasts treated for 72 h with dexamethasone and separated on 6% polyacrylamide gels in 7 M urea (see Methods). 10 μg of total RNA from control (lane *1*) and dexamethasone-treated fibroblasts (0.100 and 1.00 μM, lanes *2* and *3*, respectively) was hybridized with ^{32}P-labeled human SCF probe (*A*) where the full-length protected product of SCF is 359 nt (the second protected form of SCF of 275 nt is seen only in the control sample), or a ^{32}P-labeled human β-actin probe (*B*) where the full-length product (304 nt) and the protected product (245 nt) are visible. Densitometric analysis of SCF mRNA normalized to human β-actin mRNA in fibroblasts exposed to the indicated doses of dexamethasone is also shown (*C*).

mast cell number within these tissues (8–11, 29, 30). This decrease in mast cell number takes place over days and generally requires repeated application of glucocorticoids (8). This biologic effect of a glucocorticoid would, through the diminution of mast cell number, be expected to downregulate allergic inflammation. The mechanism by which this downregulation of mast cell number occurs is unknown.

It was our hypothesis that glucocorticoids decrease mast cell number either by modulating the synthesis of SCF within tissues, or the ability of SCF to maintain the viability of mast cells themselves, as it is known that mature mast cells require SCF for survival (19, 31). In examining the latter possibility, we found that the addition of the glucocorticoid dexamethasone to SCF-dependent mast cell cultures did not interfere with the ability of SCF to maintain the viability of these cells (Fig. 3). That is, dexamethasone did not induce mast cell apoptosis in the presence of SCF. However, our in vitro studies reproducing the reported decrease in mast cell number after the application of a topical glucocorticoid, in this case fluocinonide (Fig. 1 *A*), revealed the apoptosis of mast cells within fluocinonide-treated tissue (Figs. 1 *B* and 2, *C* and *D*). Assuming that this apoptosis was associated with a deprivation of SCF, it was then logical to hypothesize that glucocorticoids have the ability to downregulate the production of SCF from stromal cells, such as fibroblasts, which are known to produce SCF in tissues (15–18, 32). In vitro studies first revealed that dexamethasone-treated fibroblasts produced less SCF mRNA (Fig. 4) in a dose–response fashion. Furthermore, the amount of SCF protein produced by dexamethasone-treated human and mouse fibroblasts was also decreased in vitro (Fig. 5). Immunohistochemistry for SCF within fluocinonide-treated dermis similarly was interpreted as showing that fluocinonide appears to downregulate SCF within tissue (Fig. 6). Thus, both in vitro and in vivo evidence was consistent in showing that glucocorticoids downregulate SCF production. This finding is not unexpected. Glucocorticoids have been reported to decrease the production of cytokines including IL-1 and TNF (33–36).

If glucocorticoids applied to the dermis induce mast cell apoptosis through a downregulation of local SCF production by resident cells, then the administration of SCF at sites undergoing fluocinonide treatment should compensate for the decrease in local synthesis of SCF. The net result would be a normalization of tissue mast cell number. Indeed, this was the case (Fig. 7), as the administration of exogenous SCF maintained mast cell number in fluocinonide-treated dermis (Fig. 7).

In summary, these data demonstrate that the decrease in tissue mast cell number which follows the application of glucocorticoids is associated with apoptosis of mast cells. Furthermore, this apoptosis is shown to be the result of a glucocorticoid-dependent decrease in local SCF production. From these data it would be anticipated that injection of glucocorticoids

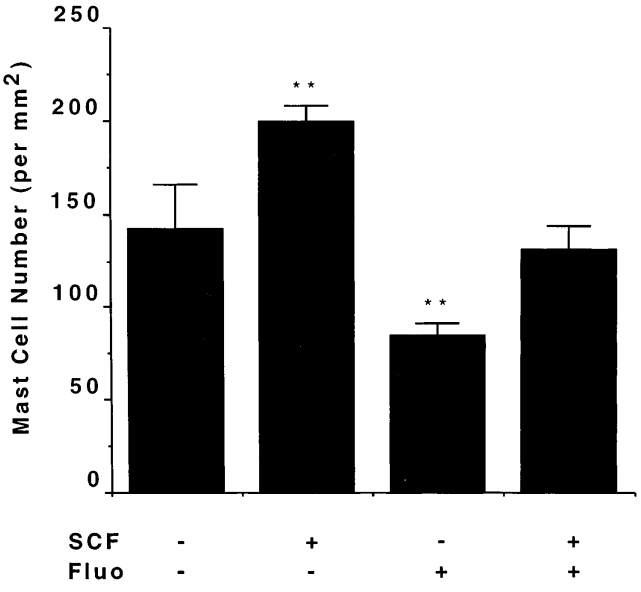


Figure 7. Effect of fluocinonide application to mouse ears on tissue mast cell number in the absence or presence of three times weekly local injections of SCF (for details, see Methods). Six sections per ear were examined. Data are presented as the mean±SEM ($n = 3$). Groups are compared with ANOVA, where $^{**}P < 0.01$.

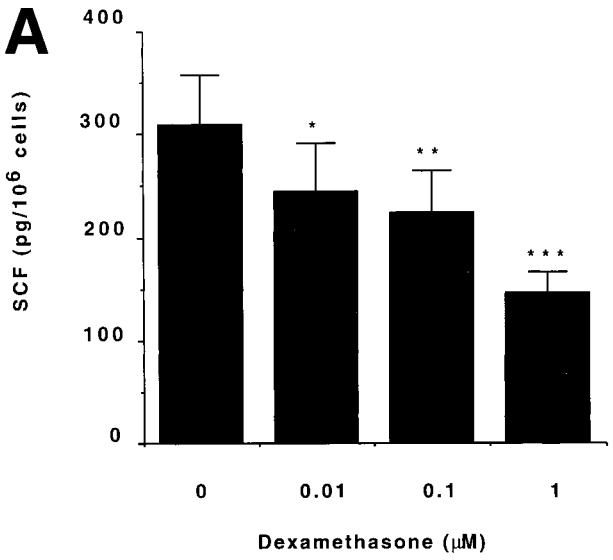
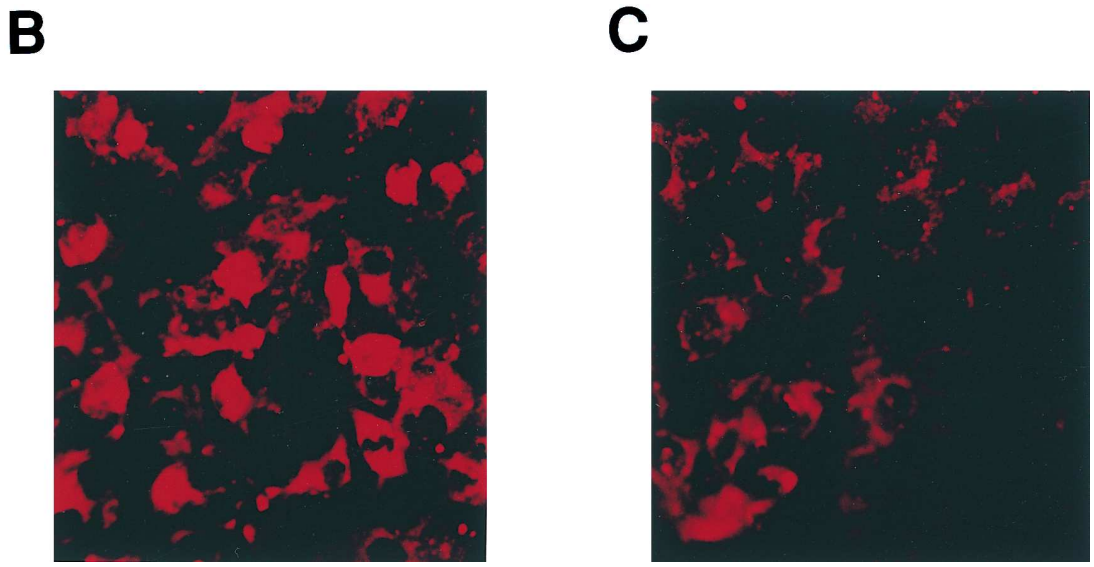


Figure 5. Inhibition of SCF protein production in fibroblasts by dexamethasone. (*A*) Human fetal skin fibroblasts were treated for 72 h with different concentrations of dexamethasone or with medium alone. Soluble SCF was measured in cell free supernatants with an ELISA assay (see Methods), data are shown as the mean±SEM ($n = 3$, each experiment performed in triplicate). Statistical analysis was performed using Student's *t* test (two-tailed) where $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. (*B* and *C*) Immunohistochemical detection of SCF protein in 3T3/NIH mouse fibroblasts (×400) after culture for 72 h without (*B*) or with (*C*) dexamethasone. Coverslips of fibroblast cultures were stained with a polyclonal antibody against SCF (10 μg/ml).



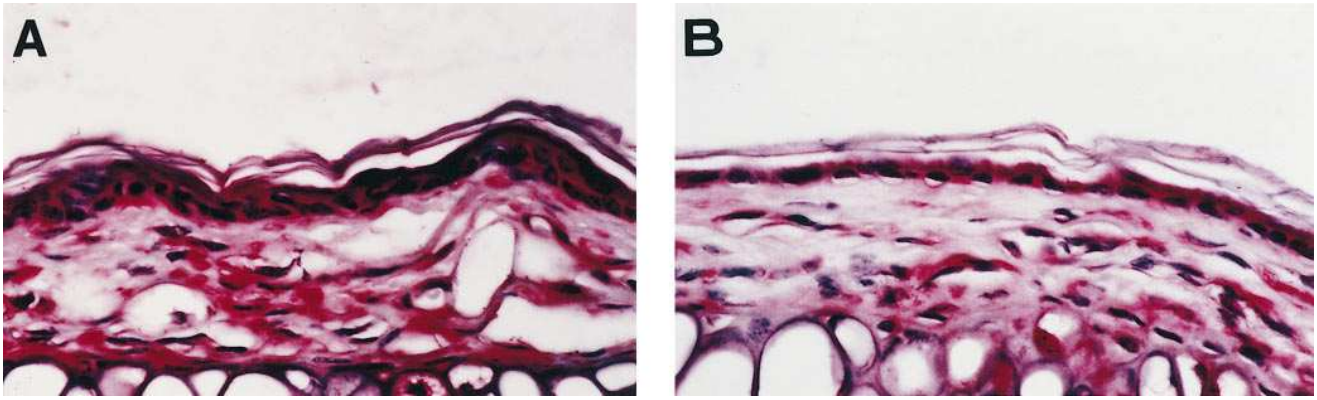


Figure 6. Immunohistochemical detection of SCF protein in mouse ear dermis. Sections (×400) of an untreated (*A*) and fluocinonide-treated ear (*B*) stained with a polyclonal antibody against SCF (10 μg/ml). Results are representative of observations using three mice in each group.

into inflamed tissues would result in a more dramatic reduction in mast cell number, as has been reported in synovial tissue after injection of glucocorticoids (37).

These data suggest that strategies based upon these observations could be developed and directed at a more efficient delivery of glucocorticoids to target stromal cells that produce SCF. This would be expected to minimize the side effect of these drugs or to maximize their ability to decrease mast cell number. Such strategies would be particularly helpful in designing new approaches to treat allergic diseases and disorders associated with mast cell hyperplasia.

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