

Dexamethasone suppresses gene expression and production of IL-13 by human mast cell line and lung mast cells

Toshiaki Fushimi, MD, Hiroshi Okayama, MD, Sanae Shimura, MD, Hiroki Saitoh, MD, and Kunio Shirato, MD Sendai, Japan

Background: IL-13 has been shown to induce IgE production in B cells by promoting class switching to IgE. Mast cells are known to play an important role in the pathogenesis of allergic diseases. We evaluated the ability of human mast cells to produce IL-13 using human mast cell line HMC-1 and freshly isolated lung mast cells and then examined the effect of dexamethasone on the gene expression and production of IL-13 by these cells. **Methods:** HMC-1 cells and lung mast cells were cultured with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μ mol/L ionomycin and with 5 μ g/ml phytohemagglutinin (PHA) and 10 ng/ml PMA, respectively, in the presence of dexamethasone. The gene expression of IL-13 at 3 hours (HMC-1 cells) or 12 hours (human lung mast cells) after stimulation was assessed semiquantitatively by sequential reverse transcription-polymerase chain reaction and Southern blot analysis. IL-13 production at 12 hours after stimulation was assayed by ELISA. **Results:** The gene expression of IL-13 by HMC-1 cells and human lung mast cells, which was detected at a low level in an unstimulated condition, was increased by PMA/ionomycin and suppressed by dexamethasone. The supernatant of HMC-1 cells and human lung mast cells showed a low level of IL-13, which was increased by the stimulation and suppressed by dexamethasone. **Conclusion:** These findings indicate that HMC-1 cells and human lung mast cells produce IL-13 and that dexamethasone suppresses the production of IL-13 by these cells through an inhibitory action on the gene expression. (*J Allergy Clin Immunol* 1998;102:134-42.)

Key words: IL-13, dexamethasone, mast cells, RNA

Allergen-specific IgE antibodies play a central role in mediating allergic reactions, including atopic bronchial asthma. IgE induction by B cells requires the cognate interaction of B cells with T cells, as well as soluble factors such as IL-4. IL-4 plays a critical role in the switching of B cells to produce IgE and may therefore be of importance in the development of atopy.^{1,2} IL-13 is a cytokine derived from T cells that is a potent modulator of human monocytes and B-cell function.³⁻⁵ IL-13 shares many properties with IL-4, which is attributable to the

Abbreviations used

AP:	Activator protein
bp:	Base pairs
PCR:	Polymerase chain reaction
PHA:	Phytohaemagglutinin
PMA:	Phorbol 12-myristate 13-acetate
SDS:	Sodium dodecylsulfate
SSPE:	Sodium sulfate phosphate ethylenediamine tetraacetic acid buffer
TNF:	Tumor necrosis factor

fact that the IL-4 and IL-13 receptor share a receptor subunit involved in signal transduction.⁶ Like IL-4, but independently of IL-4, IL-13 stimulates B-cell proliferation in the presence of CD40 ligand (CD40L), which is transiently expressed on CD4⁺ T-helper cells after activation, inducing IgE and IgG4 switching, germ line IgE heavy-chain (ϵ) transcription, and CD23 (low-affinity IgE receptor) expression.⁷⁻⁹ IL-13 also upregulates the expression of CD23 and major histocompatibility class II antigen on monocytes and downregulates the proinflammatory cytokine production by monocytes.⁵

Bronchial asthma is characterized by the presence of inflammatory cells in the bronchial mucosa, which consist of activated T cells, mast cells, and eosinophils.¹⁰⁻¹² Several studies have demonstrated a predominance of IL-4 and IL-5 mRNA expression in bronchoalveolar lavage T cells in bronchial asthma and a polarization of the cytokine profile to a T_{H2} pattern.^{13,14} Cytokines produced by T cells have been suggested to play a key role in initiating and maintaining the inflammatory infiltrate observed in the lung in both atopic and nonatopic asthma.¹⁴⁻¹⁵ Bradding et al.^{16,17} demonstrated that IL-4 was mainly produced by mast cells in asthmatic bronchial mucosa, suggesting a major role of mast cells in IgE production in bronchial asthma. Mast cells have also been considered as an important source of inflammatory cytokines,¹⁸⁻²¹ the profile of which resembles that of type 2 helper T cells. Recently, Burd et al.²² reported IL-13-like bioactivity in murine bone marrow-derived cultured mast cells and the mouse C1.MC/C57.1 mast cell line. However, we do not yet have direct evidence for the gene expression and production of IL-13 by human mast cells.

In the treatment of allergic diseases, including bronchial asthma, glucocorticoids are widely used as a most effective agent. Glucocorticoids inhibit the synthesis of several

From the First Department of Internal Medicine, Tohoku University School of Medicine, Sendai.

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Reprint requests: Kunio Shirato, MD, Professor and Chairman, First Department of Internal Medicine, Tohoku University School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan.

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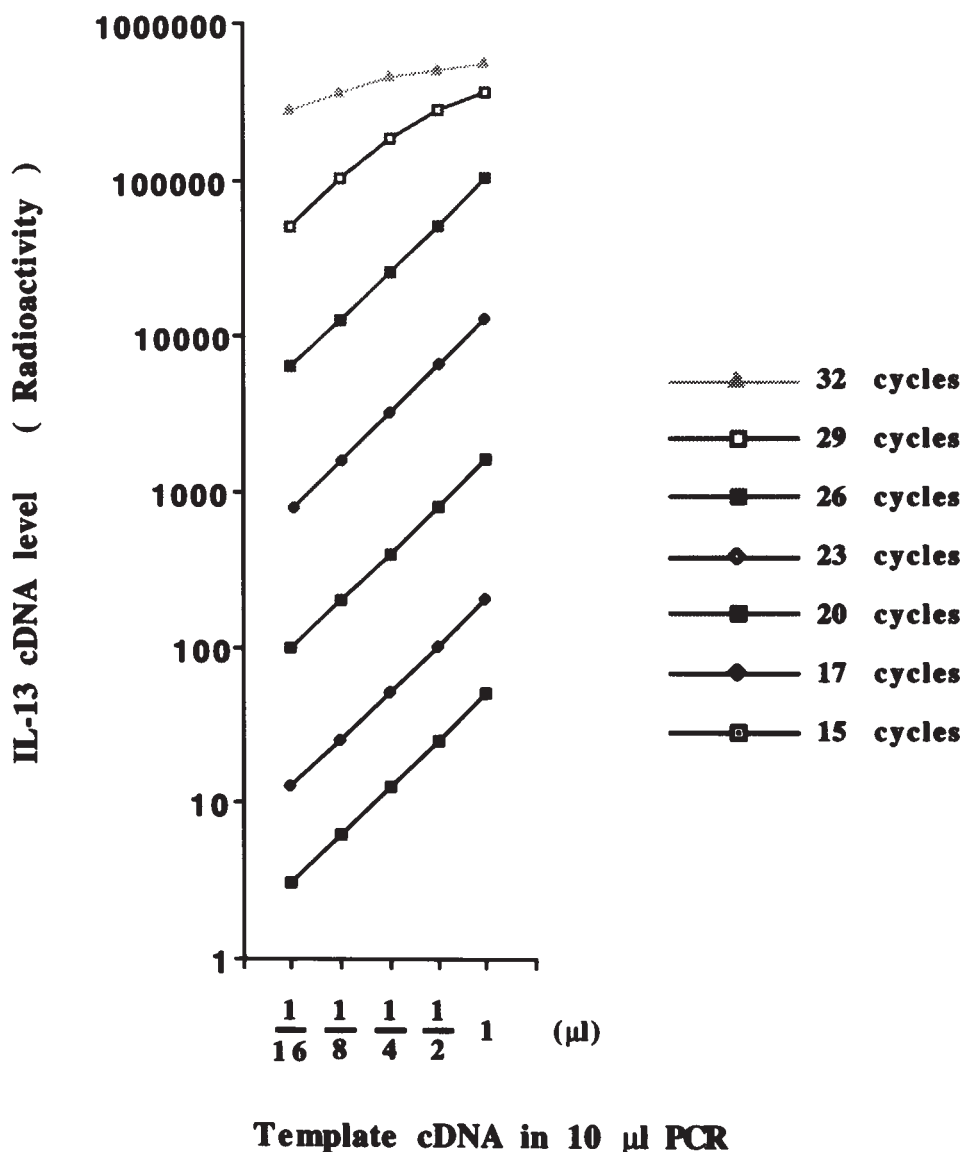


FIG. 1. Effect of number of PCR cycles on amplification of IL-13 cDNA. Different amounts of same cDNA template were amplified for 15 to 32 cycles with IL-13-specific primers. There was a linear relationship between level of amplified IL-13 cDNA fragment and amount of template at 15 to 26 cycles, but not at more than 29 cycles.

cytokines, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, granulocyte-macrophage colony-stimulating factor, interferon- γ , and tumor necrosis factor (TNF)- α and TNF- β by lymphocytes and monocytes.²³⁻³² However, some cytokines, such as macrophage colony-stimulating factor, are not suppressed by glucocorticoids.²⁹ Spahn et al.³³ reported that the expression of IL-13 was downregulated in human bronchoalveolar lavage cells after treatment with prednisone. In a mouse mast cell line, dexamethasone was reported to suppress TNF- α mRNA and activity.³⁴

The purposes of this study are to determine whether human mast cells express IL-13 gene and protein, and if so, how dexamethasone influences the gene expression and production of IL-13 in human mast cells.

METHODS

Cell preparation

Cell line. The human mast cell line HMC-1 was a generous gift from Dr. J. H. Butterfield (Mayo Clinic, Rochester, Minn.).³⁵ HMC-1 cells were maintained in Iscove's Modified Dulbecco's Medium (Gibco, Grand Island, N.Y.) supplemented with 10% (vol/vol) fetal bovine serum (Gibco), 2 mmol/L L-glutamine, 100 U/ml penicillin G, 10 mg/ml streptomycin, and 1.2 mmol/L α -thioglycerol (Sigma Diagnostics, St. Louis, Mo.). HMC-1 cells were suspended at a concentration of 5×10^5 cells/ml in the culture medium. HMC-1 cells were preincubated for 1 hour in each well of 24-well plates (Costar, Cambridge, Mass.) with or without dexamethasone (Banyu, Japan). HMC-1 cells were then incubated for up to 18 hours with the combination of 10 ng/ml phorbol 12-myristate 13-

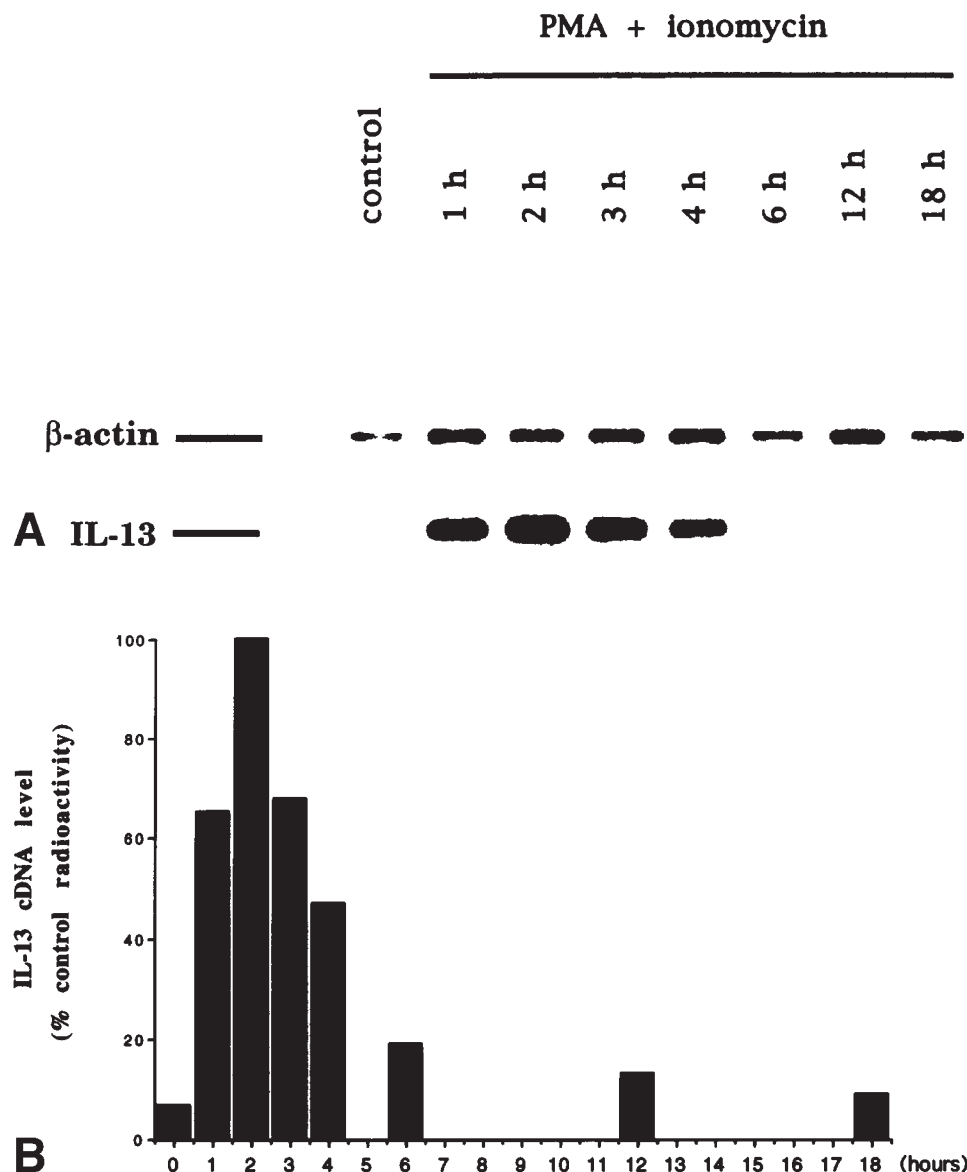


FIG. 2. Time course of IL-13 cDNA in HMC-1 cells stimulated with PMA and ionomycin. **A**, Southern blot analysis of PMA/ionomycin-stimulated HMC-1 cells. PMA/ionomycin caused rapid increase in IL-13 cDNA, which peaked at 2 hours and then decreased rapidly thereafter. **B**, Time course of IL-13 cDNA increase determined by image analyzer. Radioactivity of IL-13 cDNA band is normalized by that of β -actin cDNA band and is shown as percent of each PMA/ionomycin-stimulated value. Peak was observed between 1 and 3 hours, and it decreased thereafter.

acetate (PMA) (Sigma) and 1 μ mol/L ionomycin (Sigma) at 37° C in a humidified atmosphere of 5% CO₂ in air.

Human lung mast cells. Human lung tissue fragments were obtained from the rim of normal tissue resected during surgery for bronchogenic carcinoma. The preparation of human lung mast cells was performed by an enzymatic procedure described previously.³⁶ Briefly, the tissue was minced and subjected to enzymatic digestion with 1.5 mg/ml collagenase (Sigma) and 0.75 mg/ml hyaluronidase (Sigma). Human lung mast cells were purified by positive affinity selection with the use of an anti-c-kit monoclonal antibody YB5.B8 (Pharmingen, San Diego, Calif.). The cells were treated for 30 minutes at 4° C with the anti-c-kit monoclonal antibody YB5.B8, washed twice in Hanks

balanced salt solution (Gibco) and 2% fetal bovine serum, and then incubated for 30 minutes at 4° C with goat anti-mouse IgG Dynabeads M-450 (Dyna). The suspension was then exposed to a magnetic particle concentrator (MPC-1, Dynal) for 3 minutes. The purified cells were washed four times with the magnet. The final purities of the lung mast cells were greater than 93% as determined by toluidine blue staining. Purified mast cells were resuspended in a culture medium consisting of Dulbecco's modification of Eagle's medium (Gibco) supplemented with 10% (vol/vol) fetal bovine serum, 2 mmol/L L-glutamine, 100 U/ml penicillin G, and 10 mg/ml streptomycin. Human lung mast cells were suspended at a concentration of 1×10^6 cells/ml in the culture medium, preincubated for 1 hour in each well of 24-well

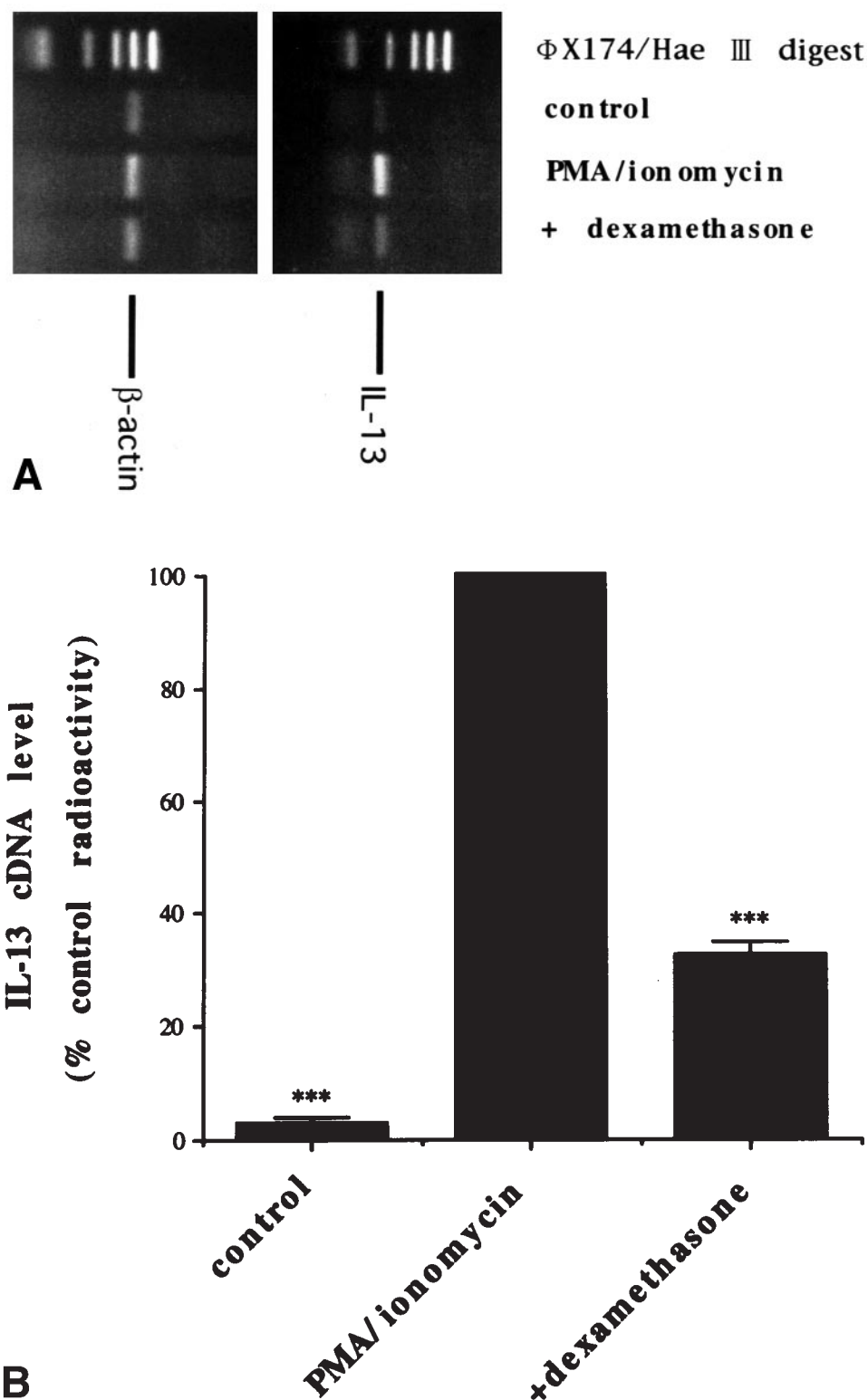


FIG. 3. The effect of dexamethasone on the PMA/ionomycin-stimulated expression of IL-13 gene in HMC-1 cells. **A**, Southern blot analysis. Length of amplified products was 501 bp and 1017 bp for IL-13 cDNA and β -actin cDNA, respectively. **B**, Effect of dexamethasone on PMA/ionomycin-stimulated expression of IL-13 gene determined by image analyzer. PMA/ionomycin significantly increased level of IL-13 cDNA, and dexamethasone significantly suppressed it by 68% at 10^{-6} mol/L. Radioactivity of IL-13 cDNA band is normalized by that of β -actin cDNA band and is shown as percent of each PMA/ionomycin-stimulated value. Each point represents mean $\pm$ SEM of four experiments. *** p < 0.001 compared with PMA/ionomycin-stimulated value.

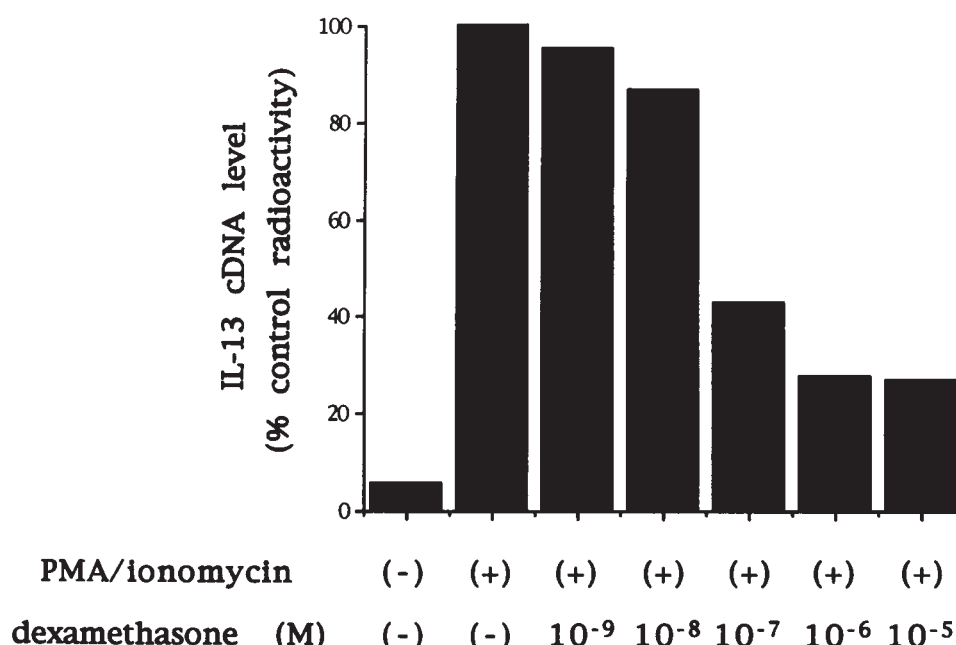


FIG. 4. Dose-response of dexamethasone on PMA/ionomycin-stimulated expression of IL-13 gene in HMC-1 cells. Radioactivity of IL-13 cDNA band is normalized by that of β -actin cDNA band and is shown as percent of each PMA/ionomycin-stimulated value. Level of IL-13 cDNA was suppressed with increasing concentrations of dexamethasone.

plates with or without dexamethasone, and then incubated for 12 hours with the combination of 10 ng/ml PMA and 1 μ M/L ionomycin at 37° C in a humidified atmosphere of 5% CO₂ in air.

Assay for IL-13 release

After culture of the HMC-1 cells and human lung mast cells for 12 hours, the cell suspensions were centrifuged and the cell-free supernatant fractions were assayed for IL-13 protein by ELISA (Bender MedSystems, Austria), which has no cross-reactivity with other proteins. The sensitivity of the assays was 1.56 pg/ml for IL-13.

Reverse transcription-polymerase chain reaction

Total RNA was isolated by lysing the cells in Isogene (Nippon gene, Japan) following the manufacturer's protocol and quantified by measurement of absorbance at 260 nm. Total RNA (0.5 μ g) was converted to first-strand cDNA with oligo-dT primer and Moloney murine leukemia virus reverse transcriptase (Gibco) according to the supplier's instructions and was then diluted to 100 μ l. The primer sequences used in polymerase chain reaction (PCR) for the IL-13 cDNA were 5'-AAGCCACCCAGCCTATGCATCC-3' and 5'-TCAATAGTCAGGTCCTGTCTCT-3', with an amplified cDNA size of 501 base pairs (bp). The primer sequences for the β -actin cDNA were 5'-ATGGGTCAGAAGGATTCCTAT-3' and 5'-TGACTTAGTTGCGTTACAC-3', with an amplified cDNA size of 1017 bp. A portion of the cDNA synthesis reaction volume ($\frac{1}{5}$) was combined at a final volume of 20 μ l for PCR amplification by using one or two sets of primers and 0.48 units of Tth DNA polymerase (Toyobo, Japan). For semiquantitative analysis of IL-13 mRNA, PCR was performed for 20 cycles, each cycle consisting of denatu-

ration at 94° C (1 minute), annealing at 54° C (1 minute), and elongation at 72° C (3 minute), which we have previously shown to be capable of semiquantitative analysis.²⁸

Southern blot analysis

The PCR-amplified products were electrophoresed on 1% agarose gels in Tris-acetic acid-ethylenediamine tetraacetic acid buffer and transferred to nylon membranes (Hybond N+; Amersham, Arlington Heights, Ill.). Hybridization was carried out in a solution with a final concentration of 5 $\times$ sodium sulfate phosphate ethylenediamine tetraacetic acid buffer (SSPE), 5 $\times$ Denhardt's solution, 0.5% sodium dodecylsulfate (SDS), 20 μ g/ml denatured salmon sperm DNA, and 5 $\times$ 10⁵ cpm/ml of ³²P-labeled IL-13 or β -actin oligonucleotide probe for 14 hours. After hybridization, the membranes were washed once in 2 $\times$ SSPE/0.1% SDS for 5 minutes, three times in 1 $\times$ SSPE/0.1% SDS at room temperature for 10 minutes, and once in 1 $\times$ SSPE/0.1% SDS at 50° C for 10 minutes. Autoradiography was performed by exposing RX film (Fuji Photo Film Co., Japan) to the washed membranes. We also exposed an imaging plate (Fuji Photo Film Co.) to the membranes. The cDNA levels were calculated on the basis of the hybridization signals on the imaging plate by a Fujix Bio-Image Analyser BAS 2000 (Fuji Photo Film Co.). The β -actin cDNA levels showed similar levels. The IL-13 cDNA levels were normalized by the β -actin cDNA levels, which are shown as percentages of each stimulated level. We evaluated the suppressive effect of dexamethasone as the rate of the suppressed IL-13 cDNA level to the level stimulated by PMA/ionomycin or phytohemagglutinin (PHA)/PMA. Oligonucleotide probes, specific for a sequence internal to the primers used in the amplification, were labeled at the 5' end by polynucleotide kinase (Gibco) and [32P]ATP (Amersham), according to

the manufacturer's recommendation. The oligonucleotides used to detect IL-13 and β -actin were 5'-CCACCTCGATTTTGGTGTCT-3' and 5'-GATGACCCAGATCATGTTTG-3', respectively. Probes were separated from nonincorporated nucleotides by passage over a sephadex G25 column (Quick Spin Column; Boehringer Mannheim, Germany).

Statistical analysis

Data are expressed as means $\pm$ SEM. For mean comparisons, one-way analysis of variance or unpaired two-tailed Student's *t* test was used. Differences were considered statistically significant at *p* values less than 0.05.

RESULTS

Quantitative analysis of IL-13 mRNA expression

We first examined whether IL-13 gene was expressed in human mast cell line HMC-1 by PCR for 30 cycles. We incubated HMC-1 cells for 12 hours in the absence or presence of the combination of 10 ng/ml PMA and 1 μ mol/L ionomycin at 37° C. The IL-13 cDNA fraction amplified band was observed in unstimulated HMC-1 cells and was increased with PMA/ionomycin stimulation by PCR, followed by electrophoresis on agarose gels with ethidium staining (data not shown). To analyze IL-13 gene expression semiquantitatively, we first determined the number of PCR cycles at which the saturation of amplification started, as we reported previously.²⁸ Briefly, the first-strand cDNA sample from which the cDNA band was mostly amplified after 26 PCR cycles was used. Different amounts of the same cDNA template were amplified for 15 to 32 cycles by using IL-13- and β -actin-specific primers. Each PCR product was electrophoresed, transferred to nylon membranes, and subjected to hybridization by using the ³²P-labeled IL-13 or β -actin oligonucleotide probe. There was a linear relationship between the level of the amplified IL-13 cDNA fragment and the amount of the template at 15 to 26 cycles, but not at more than 29 cycles (Fig. 1). Similarly, there was a linear relationship between the level of the amplified β -actin cDNA fragment and the amount of the template at 15 to 29 cycles, but not at 32 cycles (data not shown). Two independent experiments were performed with the same results. Therefore we amplified IL-13 and β -actin cDNA fragments for 20 cycles, at which the target cDNA is linearly amplified.

Kinetics of IL-13 mRNA expression by HMC-1 cells

To examine the time course of IL-13 mRNA expression by HMC-1 cells, HMC-1 cells were stimulated with 10 ng/ml PMA and 1 μ mol/L ionomycin for 1, 2, 3, 4, 6, 12, and 18 hours. Fig. 2, A shows the time course of IL-13 cDNA by PMA/ionomycin-stimulated HMC-1 cells. The IL-13 cDNA band was faintly observed in unstimulated HMC-1 cells. PMA/ionomycin caused a rapid increase in IL-13 cDNA that peaked at 2 hours and then decreased rapidly thereafter. With the use of a bio-image analyzer (BAS 2000, Fuji Photo Film Co) we calculated the intensities of IL-13 and β -actin cDNA bands. The levels of IL-13 cDNA

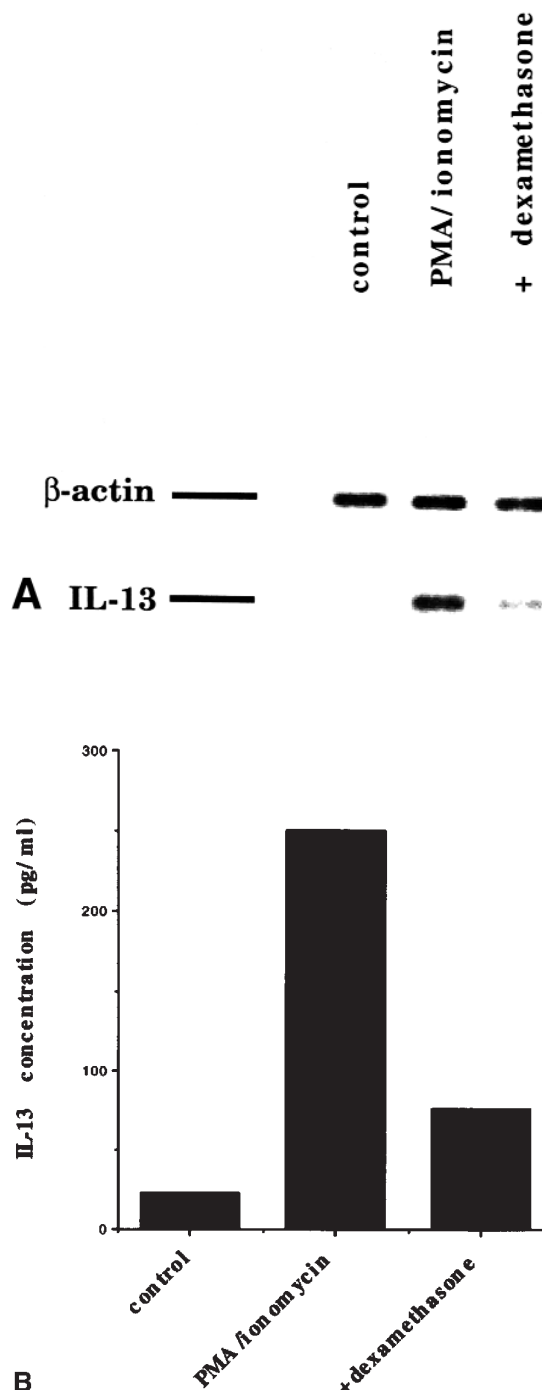


FIG. 5. A, Effect of dexamethasone on PMA/ionomycin-stimulated expression of IL-13 gene in freshly isolated human lung mast cells. IL-13 cDNA band was observed without stimulation. When stimulated with 10 ng/ml PMA and 1 μ mol/L ionomycin, thick band was identified. Intensity of band was decreased with 10^{-6} mol/L dexamethasone. **B**, Effect of dexamethasone on IL-13 production in PMA/ionomycin-stimulated human lung mast cells. Unstimulated human lung mast cells produced low level of IL-13. With PMA/ionomycin-stimulation, level of IL-13 was increased, and level was suppressed by dexamethasone. Mean value of two experiments is shown.

were normalized by the levels of β -actin cDNA. The IL-13 cDNA was detected even without stimulation. Activation of HMC-1 with PMA/ionomycin increased the level of IL-13 cDNA by 34 orders of magnitude (Fig. 2, B). The peak was observed between 1 and 3 hours and decreased thereafter. Two independent experiments were performed with similar results. Therefore for estimation of the effect of dexamethasone on IL-13 mRNA expression, we incubated HMC-1 cells for 3 hours in the presence of 10 ng/ml PMA and 1 μ mol/L ionomycin after 1 hour of preincubation with dexamethasone.

The effect of dexamethasone on IL-13 mRNA expression and production by HMC-1 cells

Fig. 3, A shows one of the autoradiographs of PMA/ionomycin-stimulated HMC-1 cells, illustrating the effect of dexamethasone. The IL-13 cDNA band was faintly observed without stimulation. When stimulated with 10 mg/ml PMA and 1 μ mol/L ionomycin, a thick band was observed, and the intensity of the band was decreased with 10^{-6} mol/L dexamethasone. As shown in Fig. 3, B, PMA/ionomycin significantly increased the level of IL-13 cDNA, and dexamethasone significantly suppressed it by 68% at 10^{-6} mol/L ($n = 4$, $p < 0.001$). The level of IL-13 cDNA was suppressed with increasing concentrations of dexamethasone. The high concentration of 10^{-5} mol/L dexamethasone showed no further suppression of IL-13 cDNA (Fig. 4).

IL-13 protein at 22 ± 2 pg/ml was observed in the supernatant of unstimulated HMC-1 cells and significantly increased to 164 ± 12 pg/ml when stimulated with PHA and ionomycin ($n = 3$, $p < 0.01$). This increased level was significantly suppressed to 77 ± 29 pg/ml with 10^{-6} mol/L dexamethasone ($n = 3$, $p < 0.05$).

The effect of dexamethasone on IL-13 mRNA expression and production by human lung mast cells

In freshly isolated human lung mast cells, a faint band of IL-13 cDNA was observed without stimulation. When stimulated with PMA and ionomycin, the intensity of the band was increased, and it was decreased with 10^{-6} mol/L dexamethasone (Fig. 5, A).

A low level of IL-13 protein (23 pg/ml) was identified in the supernatant of human lung mast cells. When stimulated with PHA and ionomycin, human lung mast cells produced 250 pg/ml of IL-13, which was suppressed to 75 pg/ml (Fig. 5, B). These concentrations are the mean values of two experiments.

DISCUSSION

In these experiments we demonstrated that the IL-13 gene was expressed in stimulated HMC-1 cells and freshly isolated lung mast cells with PMA/ionomycin and was also detected at a low level even without stimulation. Furthermore, we showed IL-13 pro-

duction by HMC-1 cells and freshly isolated lung mast cells. Although Burd et al.²² reported IL-13-like bioactivity in murine bone marrow-derived cultured mast cells and the mouse C1.MC/C57.1 mast cell line, the specificity of IL-13-like bioactivity was not confirmed by a neutralizing experiment with anti-IL-13 antibody. We definitely demonstrated IL-13 production by HMC-1 cells and human lung mast cells using sandwich ELISA, which has no cross-reactivity with other proteins. Mast cells were shown to not only produce IL-4 but also to express CD40L. They were also shown to induce IgE production by B cells even in the absence of T cells.³⁷ Our experiment demonstrated that human mast cells produce IL-13, suggesting a possible role of mast cells to induce IgE by producing IL-13, as well as IL-4.

The kinetic study indicated that the increase in IL-13 mRNA could be detected 1 hour after PMA/ionomycin stimulation, reaching its maximum at between 1 and 3 hours and then falling rapidly. Zurawski and de Vries³⁸ reported that IL-13 mRNA expression in stimulated T cells persisted at high levels through 72 hours after stimulation. We observed that the IL-13 mRNA level in peripheral blood mononuclear cells at 12 hours after stimulation by PHA/PMA did not differ from that at 3 hours (unpublished data). This is in accordance with their observation. However, the time course of IL-13 mRNA in HMC-1 cells when stimulated with PMA/ionomycin was different from that in T cells. This may have resulted from the difference in culture conditions, including stimulation or a different mechanism of IL-13 mRNA expression.

Next, we demonstrated glucocorticoid inhibition of IL-13 gene expression and production by HMC-1 cells and lung mast cells. In a mouse mast cell line, it was reported that dexamethasone suppressed TNF- α mRNA and its activity.³⁴ Our experiments using human mast cell line HMC-1 and lung mast cells indicated that dexamethasone suppressed IL-13 gene expression in these cells. Dexamethasone suppressed the level of IL-13 cDNA by PMA/ionomycin-stimulated HMC-1 cells in a dose-dependent manner. Almost maximum suppression was achieved at 10^{-6} mol/L. The higher concentration of 10^{-5} mol/L dexamethasone showed no further suppression of IL-13 cDNA. The concentration that would result in 50% inhibition was calculated to be 7×10^{-8} mol/L. In these experiments, dexamethasone partially suppressed IL-13 mRNA and had less effect on the production of IL-13. The levels of IL-13 mRNA and IL-13 protein were assessed at 3 and 12 hours after stimulation, respectively, to get these maximal reactions. The former means the transient levels of IL-13 mRNA at 3 hours, and the latter means the cumulative amount of IL-13 protein secreted for 12 hours. The smaller effect on the production of IL-13 may come from this difference in incubation time. It is particularly important that IL-13 gene expression was significantly suppressed by dexamethasone in a dose range of 10^{-8} to 10^{-6} mol/L, which was similar to that

which might be achieved after therapeutic administration of corticosteroids.³⁹⁻⁴¹

Glucocorticoid receptor complex inhibits the transcription of cytokine genes through direct binding to the glucocorticoid response elements or through antagonism of the action of transcription factors responsible for mediating cytokine expression, such as activator protein-1 (AP-1), nuclear factor- κ B (NF- κ B), and nuclear factor of activated cell (NF-AT).²³ A candidate AP-1 binding site was reported to be located in the promotor region of the human and mouse IL-13 gene.⁴² Therefore it is expected that glucocorticoids directly suppress IL-13 gene transcription by inhibiting the transcriptional activity of AP-1. However, further mechanisms, such as the decrease in the stability of IL-13 mRNA, cannot be ruled out. The decrease in the stability of IL-1 β mRNA and IL-2 mRNA by glucocorticoids has been reported.^{25,43} Because dexamethasone suppressed the gene expression of IL-13 more strongly than the production, the inhibition of IL-13 gene expression is likely the most important process in the inhibition of IL-13 production. However, other processes occurring at the translational and secretional levels may further contribute to controlling IL-13 production. It is an important step for the next study to elucidate the molecular mechanisms of the suppressive effect of glucocorticoid on IL-13 gene expression and to determine the possible role that the suppression of IL-13 production by glucocorticoids may have in the treatment of allergic diseases.

In conclusion, this report indicates that human mast cells produce IL-13 and that glucocorticoids suppress the gene expression and production of IL-13 by human mast cells. These findings suggest that mast cells play a role in allergic inflammation by producing IL-13 and that the suppression of IL-13 production by glucocorticoids is one of the mechanisms by which glucocorticoids suppress allergic inflammation in the treatment of allergic diseases, including bronchial asthma.

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REFERENCES

- Romagnani S. Regulation and dysregulation of human IgE synthesis. *Immunol Today* 1990;11:316-21.
- de Vries JE, Gauchat J-F, Aversa GG, Punnonen J, Gascan H, Yssel H. Regulation of IgE synthesis by cytokines. *Curr Opin Immunol* 1991;3:851-8.
- Minty A, Chalon P, Derocq JM, Dumont X, Guillemot JC, Kaghad M, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature* 1993;362:248-50.
- McKenzie AN, Culpepper JA, de Waal Malefyt R, Briere F, Punnonen J, Aversa G, et al. Interleukin-13, a novel T cell-derived cytokine that regulates monocyte and B cell function. *Proc Natl Acad Sci USA* 1993;90:3735-9.
- de Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Benett B, Culpepper J, et al. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. *J Immunol* 1993;151:6370-81.
- Zurawski SM, Vega F Jr, Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *EMBO J* 1993;12:2663-70.
- Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin-13 induces interleukin-4-independent IgG4 and IgE 1993;90:3730-4.
- Punnonen J, de Vries JE. IL-13 induces proliferation, Ig isotype switching, and Ig synthesis by immature human fetal B cells. *J Immunol* 1994;152:1094-102.
- Cocks B, de Waal Malefyt R, Galizzi J-P, de Vries JE, Aversa G. IL-13 induces proliferation and differentiation of human B cells activated by the CD40 ligand. *Int Immunol* 1993;5:657-63.
- Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis* 1993;147:697-704.
- Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, et al. Identification of activated T-lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990;142:1407-13.
- Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988;137:62-9.
- Robinson DS, Hamid Q, Ying S, Tscipoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
- Robinson DS, Ying S, Bentley AM, Meng Q, North J, Durham SR, et al. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J Allergy Clin Immunol* 1993;92:397-403.
- Gonzalez MC, Diaz P, Galleguillos FR, Ancic P, Cromwell O, Kay AB. Allergen-induced recruitment of bronchoalveolar (OKT4) and suppressor (OKT8) cells in asthma: relative increases in OKT8 cells in single early responders compared with those in late-phase responders. *Am Rev Respir Dis* 1987;136:600-4.
- Bradding P, Okayama Y, Howarth PH, Church MK, Holgate ST. Heterogeneity of human mast cells based on cytokine content. *J Immunol* 1995;155:297-307.
- Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, 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;10:471-80.
- Plaut M, Pierce JH, Watson CJ, Hanly-Hyde J, Nordan RP, Paul WE, et al. Mast cell line produce lymphokines in response to cross-linkage of Fc ϵ RI or to calcium ionophores. *Nature* 1989;339:64-7.
- Wodnar-Filipowicz A, Heusser CH, Moroni C. Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. *Nature* 1989;339:150-2.
- Moller A, Lippert U, Lessmann D, Kolde G, Hamann K, Welker P, et al. Human mast cells produce IL-8. *J Immunol* 1993;151:3261-6.
- Burd PR, Rogers HW, Gordon JR, Martin CA, Jayaraman S, Wilson SD, et al. Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J Exp Med* 1989;170:245-57.
- Burd PR, Thompson WC, Max EE, Mills FC. Activated mast cells produce interleukin 13. *J Exp Med* 1995;181:1373-80.
- Wassim YA, Hayfa NB, Amal AR, Michael JR. Regulation of cytokine and cytokine receptor expression by glucocorticoids. *J Leukoc Biol* 1996;60:563-72.
- Lew W, Oppenheim JJ, Matsushima K. Analysis of the suppression of IL-1 α and IL-1 β production in human peripheral blood mononuclear adherent cells by a glucocorticoid hormone. *J Immunol* 1988;140:1895-902.
- Boumpas DT, Anastassiou ED, Older SA, Tsokos GC, Nelson DL, Balow JE, et al. Dexamethasone inhibits human IL-2 but not IL-2 receptor gene expression in vitro at the level of nuclear transcription. *J Clin Invest* 1991;87:1739-47.
- Culpepper JA, Lee F. Regulation of IL-3 expression by glucocorticoids in cloned murine T lymphocytes. *J Immunol* 1985;135:3191-7.
- Wu CY, Fargeas C, Nakajima T, Delespesse G. Glucocorticoids suppress the production of interleukin 4 by human lymphocytes. *Eur J Immunol* 1991;21:2645-7.
- Okayama H, Fushimi T, Shimura S, Sasaki H, Shirato K. Glucocorticoids suppressed production and gene expression of interleukin 5 by peripheral blood mononuclear cells in atopic patients and normal sub-

- jects. *J Allergy Clin Immunol* 1994;93:1006-12.
29. Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M, Fey MF. Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8 and IL-6, but not of M-CSF, in human fibroblasts. *Blood* 1992;79:45-51.
30. Fushimi T, Okayama H, Seki T, Shimura S, Shirato K. Dexamethasone suppressed gene expression and production of interleukin-10 by human peripheral blood mononuclear cells and monocytes. *Int Arch Allergy Immunol* 1997;112:13-8.
31. Arya SK, Wong-Staal F, Gallo RC. Dexamethasone-mediated inhibition of human T cell growth factor and gamma-interferon mRNA. *J Immunol* 1984;133:273-6.
32. Waage A, Bakke O. Glucocorticoids suppress the production of tumor necrosis factor by lipopolysaccharide-stimulated human monocytes. *Immunology* 1988;63:299-302.
33. Spahn JD, Hamid Q, Surs W, Szeffler, Leung DYM. IL-13 mRNA expression in steroid-resistant asthma: diminished glucocorticoid receptor binding affinity is induced by IL-13 [abstract]. *J Allergy Clin Immunol* 1995;95:385.
34. Wershil BK, Furuta GT, Lavigne JA, Choudhury AR, Wang Z-S, Galli SJ. Dexamethasone or cyclosporin A suppress mast cell-leukocyte cytokine cascades. *J Immunol* 1995;154:1391-8.
35. Butterfield JH, Weiler D, Dewald G, Gleich GJ. Establishment of immature mast cell line from a patient with mast cell leukemia. *Leukemia Res* 1988;12:345-55.
36. Okayama Y, Hunt TC, Kassel O, Ashman LK, Church MK. Assessment of the anti-c-kit monoclonal antibody YB5.B8 in affinity magnetic enrichment of human lung mast cells. *J Immunol Methods* 1994;169:153-61.
37. Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993;365:340-3.
38. Zurawski G, de Vries JE. Interleukin-13, an interleukin-4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol Today* 1994;15:19-26.
39. Tsuei SE, Moore RG, Ashley JJ, McBride WG. Disposition of synthetic glucocorticoid. I. Pharmacokinetics of dexamethasone in healthy adults. *J Pharmacokinet Biopharm* 1979;7:249-63.
40. Loew D, Schuster O, Graul EH. Dose-dependent pharmacokinetics of dexamethasone. *Eur J Clin Pharmacol* 1986;30:225-30.
41. Young MC, Cook N, Read GF, Hughes IA. The pharmacokinetics of low-dose dexamethasone in congenital adrenal hyperplasia. *Eur J Clin Pharmacol* 1989;37:75-7.
42. McKenzie ANJ, Li X, Largaespada DA, Sato A, Kaneda A, Zurawski SM, et al. Structural comparison and chromosomal localization of the human and mouse IL-13 genes. *J Immunol* 1993;150:5436-44.
43. Lee SW, Tsou A, Chan H, Thomas J, Pettie K, Eugui EM, et al. Glucocorticoids selectively inhibit the transcription of the interleukin gene and decrease the stability of interleukin 1b mRNA. *Proc Natl Acad Sci USA* 1988;85:1204-8.

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