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Short Report

Suppression of Gene Expression and Production of Interleukin 13 by Dexamethasone in Human Peripheral Blood Mononuclear Cells

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Fushimi, T., Shimura, S., Suzuki, S., Saitoh, H., Okayama, H. and Shirato, K. *Suppression of Gene Expression and Production of Interleukin 13 by Dexamethasone in Human Peripheral Blood Mononuclear Cells.* Tohoku J. Exp. Med., 1998, **185** (2), 157-160 —— We examined the effect of dexamethasone on the gene expression and production of interleukin (IL)-13 by human peripheral blood mononuclear cells from healthy controls. The gene expression was assessed semiquantitatively by sequential transcription polymerase chain reaction and Southern blot analysis, and the production of this cytokine was assessed by enzyme-linked immunosorbent assay (ELISA). Dexamethasone suppressed IL-13 gene expression induced by stimulation with phytohemagglutinin and phorbol 12-myristate 13-acetate in a dose-dependent manner, with 96% suppression at 10^{-6} M, and also suppressed the increased production of IL-13. This is suggested to be one of the mechanisms by which glucocorticoids suppress allergic inflammation.
—— Interleukin 13; dexamethasone; human mononuclear cells; messenger RNA © 1998 Tohoku University Medical Press

Interleukin (IL)-13 is a cytokine derived from T cells and shares many properties with IL-4. However, IL-13 is able to independently induce class switching and IgE secretion from human B cells, and further, IL-13 upregulates the expression of CD23 (low affinity IgE receptor) and major histocompatibility complex (MHC) class II antigen on monocytes (McKenzie et al. 1993). Bronchial asthma is characterized by the accumulation of inflammatory cells (activated T cells, mast cells and eosinophils) in the airways, and cytokines produced by T cells have been suggested to play a key role in initiating and maintaining the airway inflammation in both atopic and non-atopic asthma. Although glucocorticoid downregulates other cytokine productions by T helper lymphocytes (Arya et al. 1984; Culpepper and Lee 1985), we have little knowledge concerning the effect of

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glucocorticoid on IL-13 gene expression or production by peripheral blood mononuclear cells (PBMCs) or T-cells.

Materials and Methods

PBMCs were isolated from venous blood obtained from 5 normal healthy subjects (all nonsmokers and non-atopic, a female and 4 males, 30 ± 4 years) by gradient centrifugation, as previously reported (Okayama et al. 1994; Fushimi et al. 1997). PBMCs were suspended at a concentration of 5×10^6 cells/ml in the culture medium (Okayama et al. 1994; Fushimi et al. 1997), and incubated for 12 hours with 5 μg/ml phytohemagglutinin (PHA) and 10 ng/ml phorbol 12-myristate 13-acetate (PMA) at 37°C in a humidified atmosphere of 5% CO_2 in air after 1 hour- preincubation with or without dexamethasone.

After culture of PBMCs for 12 hours, the cell suspensions were centrifuged and the cell free supernatant fractions were assayed for IL-13 protein by enzyme-linked immunosorbent assay (ELISA), as previously reported (Fushimi et al. 1998).

For reverse transcription-polymerase chain reaction (RT-PCR), total RNA (0.5 μg) isolated by lysing the cells was converted to first strand cDNA. The primer sequences used in PCR for the IL-13 cDNA and β-actin cDNA were described in a previous report (Fushimi et al. 1998). For the quantitative analysis of IL-13 mRNA, PCR was performed for 20 cycles, each cycle consisting of denaturation at 94°C (1 minutes), annealing at 54°C (1 minutes) and elongation at 72°C (3 minutes), as previously described (Fushimi et al. 1998).

For Southern blot analysis, the cDNA levels were calculated on the basis of the hybridization signals by a Fujix Bio-Image Analyser BAS 2000 (Fuji Photo Film Co., Tokyo), and normalized by the β-actin cDNA levels. The oligonucleotides used to detect IL-13 and β-actin were described in a previous report (Fushimi et al. 1998).

Results

In the autoradiographs of Southern blotting, the IL-13 cDNA band was faintly observed without stimulation, and stimulation with PHA/PMA induced a thick band that was decreased with increasing concentrations of dexamethasone. A representative example is shown in Fig. 1A. As shown in Fig. 1B, the quantitative analysis showed that PHA/PMA significantly increased the level of IL-13 cDNA, and dexamethasone significantly suppressed it in a dose-dependent manner, with 96% suppression at 10^{-6} M. The concentration that results in 50% inhibition (IC_{50}) was calculated to be 3×10^{-8} M.

Unstimulated PBMCs produced 172 ± 79 pg/ml of IL-13 ($n=5$). PHA/PMA-stimulated PBMCs produced 1439 ± 191 pg/ml of IL-13 ($n=5$, $p<0.01$, two-tailed Student's t-test), and 10^{-6} M dexamethasone suppressed IL-13 production to 827 ± 106 pg/ml ($n=5$, $p<0.05$).

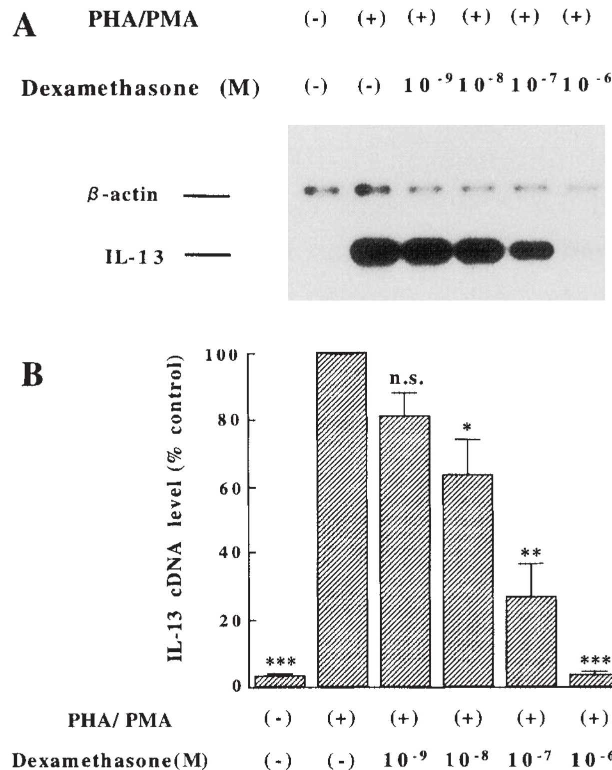

Fig. 1. A: A representative example of Southern blot analysis of IL-13 gene expression in PBMCs. The length of the amplified products was 501 bp and 1017 bp, for IL-13 and β-actin cDNA, respectively. When stimulated with PHA/PMA, a thick band was identified. The intensity was decreased with increasing concentrations of dexamethasone.
B: Effect of dexamethasone on the IL-13 gene expression by PBMCs. PHA/PMA significantly increased the level of IL-13 cDNA. Dexamethasone significantly suppressed it in a dose-dependent manner with 96% suppression at 10^{-6} M. The radioactivity of IL-13 cDNA band is normalized by that of β-actin cDNA band and is shown as a percentage of each PHA/PMA-stimulated value. Each point represents the mean$\pm$SEM of 5 experiments. *n.s.*, not significant; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared with PHA/PMA-stimulated value (Student's *t*-test)

Discussion

In the present study, we used RT-PCR for the quantitative analysis of IL-13 rather than Northern blotting because there had been a possibility that the mRNA level in PBMCs was too low to be detected by the Northern blotting. Further, our previous experiments have revealed the quantitative analysis of the present RT-PCR method for IL-13 (Fushimi et al. 1998) as well as IL-5 (Okayama et al. 1994) and IL-10 (Fushimi et al. 1997).

We showed that dexamethasone suppressed IL-13 gene expression by PBMCs in a dose-dependent manner. The suppressive rate of 10^{-6} M dexamethasone was 96% with 3×10^{-8} M of IC_{50} in PBMCs, which is higher than the 68% with 7×10^{-8} M of IC_{50} in a human mast cell line in the same manner in the present experiment (Fushimi et al. 1998), suggesting a difference between mast cells and

PBMCs. The effect of glucocorticoids also seems to have differences in gene expression among cytokines; dexamethasone similarly suppressed IL-5 (Okayama et al. 1994) and IL-13 gene expression by PBMCs as shown in this paper, while it induced only a partial inhibition in IL-10 gene expression by PBMCs (Fushimi et al. 1997). It should be noted that IL-13 gene expression by PBMCs was significantly suppressed by dexamethasone in a dose range of 10^{-8} to 10^{-6} M, which is similar to therapeutic concentrations.

During the preparation of this paper, Braun et al. (1997) also reported the inhibition of IL-13 gene expression by dexamethasone in human PBMCs. However, in their experiment, statistical analysis was not done and the dose-dependency was not clear. Further, we stimulated PBMCs with PHA/PMA, whereas they used antigen-driven PBMCs. Glucocorticoid receptor complex is known to inhibit the transcription of other cytokine genes through factors such as AP-1, NF-κB and NF-AT. Taken together with their result, the present finding supports this idea in the IL-13 gene expression by PBMCs as well as by human mast cells (Fushimi et al. 1998).

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