

Inhibitory effect of lidocaine on T cells from patients with allergic asthma

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Background: Nebulized lidocaine enables dosages of oral corticosteroids to be tapered in the treatment of severe asthma.

Objective: We sought to investigate the effect of lidocaine on T cells from patients with allergic asthma.

Methods: PBMCs and CD4⁺ T cells were isolated from 6 patients with asthma and house dust mite allergy. PBMCs were cultured with lidocaine for up to 7 days and then stained with propidium iodide to evaluate the involvement of apoptosis. In addition, the viability of CD4⁺ T cells when cultured with lidocaine was investigated. Effects of lidocaine on proliferative response, mRNA expression, and protein production of IL-5 and IFN- γ by PBMCs were investigated after stimulation with *Dermatophagoides farinae*, purified protein derivative, and phorbol 12-myristate 13-acetate plus calcium ionophore. The effects of lidocaine on the proliferative response of steroid-insensitive PBMCs from 6 nonallergic donors induced by preculture with IL-2 and IL-4 were also investigated.

Results: No significant increase in the staining of PBMCs with propidium iodide was observed in the presence of 100 μ mol/L lidocaine. The viability of CD4⁺ T cells was not significantly affected by culture with lidocaine at this concentration. However, lidocaine inhibited, in a dose-dependent manner, the proliferative response and mRNA expression and protein production of IL-5 and IFN- γ of PBMCs stimulated with *D farinae*, purified protein derivatives, or phorbol 12-myristate 13-acetate plus calcium ionophore. Preincubation of PBMCs with IL-2 and IL-4 significantly decreased the inhibitory effects of both corticosteroids and lidocaine compared with that after preincubation with medium alone.

Conclusion: Lidocaine has immunoregulatory effects on T cells. Therefore, lidocaine might be studied as an anti-inflammatory agent in the treatment of severe steroid-dependent asthma. (J Allergy Clin Immunol 2002;109:485-90.)

Key words: Asthma, lidocaine, T cell, IL-5, IFN- γ , apoptosis, steroid

Bronchial asthma is characterized by persistent airway inflammation induced by several types of inflammatory cells, including CD4⁺ T cells, mast cells, and eosinophils.¹⁻³ Because airway inflammation is closely

Abbreviations used

PI: Propidium iodide

PMA: Phorbol 12-myristate 13-acetate

PPD: Purified protein derivative

associated with airway hyperresponsiveness, airway limitation, and asthma symptoms, inhaled corticosteroids are now first-line anti-inflammatory agents for the treatment of asthma.⁴ Although asthma is often well controlled with inhaled corticosteroids, some patients with severe asthma require additional oral corticosteroids. Long-term use of oral corticosteroids often leads to such side effects as adrenal suppression, growth suppression, and osteoporosis. Therefore, steroid-sparing alternatives to oral corticosteroids are needed.⁵

Lidocaine is widely used as a short-acting local anesthetic and as an anti-arrhythmic agent. Nebulized lidocaine attenuates bronchoconstriction induced by histamine, hyperosmolar saline, and exercise in patients with asthma.⁶⁻⁸ Recent preliminary studies in adults and children with severe asthma have demonstrated that nebulized lidocaine enables oral corticosteroids to be reduced in dosage or eliminated in long-term users.^{9,10} These studies suggest that nebulized lidocaine has anti-inflammatory effects.

Lidocaine has been shown to inhibit the function of mast cells and eosinophils in vitro. Lidocaine directly inhibits histamine release from mast cells, partly by a decrease in calcium mobilization.¹¹ Furthermore, recent studies by Ohnishi et al¹² and Okada et al¹³ have demonstrated that lidocaine and its derivatives promote apoptosis of eosinophils. Although T_H2 cells play an important role in asthma, the effect of lidocaine on T cells remains unclear.

In the present study, we examined the effects of lidocaine on proliferative response and cytokine production by T cells from patients with allergic asthma after allergen-specific and non-allergen-specific stimulation. Furthermore, the effect of lidocaine on steroid-insensitive T cells was analyzed.

METHODS

Reagents

The following reagents were used: hydrochloride lidocaine, phorbol 12-myristate 13-acetate (PMA), dexamethasone (Sigma, St Louis, Mo), calcium ionophore (Calbiochem, La Jolla, Calif), recombinant (r) IL-2 (donated by Shionogi Pharmaceuticals Co, Osaka, Japan), and rIL-4 (R & D Systems, Minneapolis, Minn).

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Subjects

Six nonsmoking patients with allergic asthma were selected on the basis of (1) positive cutaneous reactions to house dust mite extract, (2) high levels of house dust- and *Dermatophagoides farinae*-specific IgE in sera, (3) baseline FEV₁ of more than 70% of the predicted value, (4) increased bronchial responsiveness to histamine, and (5) no treatment other than rescue β_2 -agonists for at least 3 months. No subjects had respiratory infections in the previous 4 weeks. Six normal donors were also investigated. Informed consent was obtained before the study.

Purification of PBMCs and CD4⁺ T cells

PBMCs were isolated from heparinized venous blood on Ficoll-Hypaque gradients (Nacalai Tesque Co, Kyoto, Japan) and then separated into T cells and non-T cells with a sheep erythrocyte-rosette technique. A CD4⁺ T-cell subset was purified from T cells by means of negative selection by using panning with OKT8 mAb (Ortho Pharmaceuticals, Raritan, NJ). In all experiments cells were cultured in RPMI-1640 supplemented with 10% vol/vol human AB serum, 100 U of penicillin, and 100 μ g/mL streptomycin in an atmosphere containing 5% CO₂ at 37°C. To investigate its effect on T cells, lidocaine (1, 10, 100, and 1000 μ mol/L) was added to the culture medium.

Flow-cytometric analysis of apoptosis

PBMCs were cultured in the absence or presence of lidocaine in the medium for 7 days. Cells were stained with propidium iodide (PI). The samples were analyzed with a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Sunnyvale, Calif).

CD4⁺ T-cell survival assay

The survival assay of CD4⁺ T cells (1×10^6 cells/well) was performed for up to 7 days in the presence or absence of lidocaine. CD4⁺ T cells were stained with trypan blue, and both live cells and total cells were counted. Percentages of viable cells were calculated by dividing the number of live cells by the total cell number. Changes in the percentages of total cell numbers and viable cells were calculated on the basis of the number of cells added to the culture on day 0.

Proliferation assay and cytokine production

For the proliferation assay, PBMCs (1×10^5 cells/well) were cultured with 10 μ g/mL *D farinae* or 5 μ g/mL purified protein derivative (PPD) antigens in 96-well plates for 7 days immediately after purification. Cells were pulsed with 1 μ Ci of tritiated methylthymidine (Amersham Corp, Arlington Heights, Ill) for the last 8 hours of the culture period. The stimulation index was calculated by dividing the counts per million of allergen-stimulated cultures by that of unstimulated cultures. For cytokine production, PBMCs (1×10^6 cells) were either activated with 10 μ g/mL *D farinae* or 5 μ g/mL PPD antigens for 72 hours or not activated. Secretion of IL-5 and IFN- γ in the supernatant was measured with ELISA kits (Biosource International, Camarillo, Calif). The ELISA kits could detect IL-5 and IFN- γ at concentrations as low as 10 pg/mL.

RT-PCR analysis

PBMCs (1×10^6 cells) were stimulated with *D farinae* or PPDs for 24 hours and with PMA plus calcium ionophore for 8 hours. Total cellular RNA was separated with the guanidinium isothiocyanate extraction and cesium chloride centrifugation method. The first-strand cDNA kit (SuperScript Preamplication System; Life Technologies, Paisley, Scotland, United Kingdom) was used to produce cDNA. Each cDNA was amplified with a SuperTaq Premix kit (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom)

by using specific primers for IL-4, IL-5, IFN- γ , and β -actin (all from Clontech, Palo Alto, Calif) with 1 μ g of total RNA. Thermal cycling of these cytokines was performed as follows: denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 120 seconds. PCR products were analyzed with electrophoresis through 2% agarose gels and visualized under UV light after ethidium bromide staining.

Establishment of steroid-insensitive PBMCs in vitro

Steroid resistance was induced with a method reported previously.¹⁴ Briefly, freshly isolated PBMCs from healthy donors were cultured in the presence of IL-2 (50 U/mL) and IL-4 (50 U/mL) for 48 hours. Cells were then washed, stimulated with PMA plus calcium ionophore for an additional 72 hours, and pulsed with 1 μ Ci of tritiated methylthymidine for the last 8 hours of the culture period. In some experiments cells were stimulated with PMA plus calcium ionophore in the presence of dexamethasone or lidocaine.

Statistical analysis

The significance of differences between groups was examined with the Wilcoxon rank test. Data are expressed as means $\pm$ SEM, and a *P* value of less than .05 was considered to indicate significance.

RESULTS

Cell viability

Because a recent study has demonstrated that lidocaine and its derivatives can promote apoptosis of eosinophils,¹³ we studied the effect of lidocaine on T cells. The percentage of PI-stained cells after incubation of PBMCs for 7 days was $13.8\% \pm 2.25\%$ with 10 μ mol/L lidocaine, $16.2\% \pm 2.40\%$ with 100 μ mol/L lidocaine, and $27.3\% \pm 2.97\%$ with 1000 μ mol/L lidocaine. We next studied the direct effect of lidocaine on the viability of CD4⁺ T cells. The total number of cells was counted, and cell viability was analyzed by staining with trypan blue. Although lidocaine at concentrations as high as 100 μ mol/L did not affect either total cell number or cell viability, lidocaine at 1000 μ mol/L decreased both the total cell number and cell viability of CD4⁺ T cells (*P* < .01, Fig 1). Because lidocaine at 1000 μ mol/L increased the staining of PBMCs with PI and decreased both the total cell number and the viability of CD4⁺ T cells, subsequent experiments were performed with concentrations of lidocaine as high as 100 μ mol/L.

Proliferative response

Proliferative responses increased when PBMCs from patients with allergic asthma were stimulated with either *D farinae* or PPD allergens. Although lidocaine had no effect on resting cell numbers, it significantly inhibited proliferative responses after allergen stimulation (*P* < .05, Fig 2). Furthermore, lidocaine inhibited proliferative responses stimulated with PMA and calcium ionophore (*P* < .05, Fig 2). In other experiments we found that the proliferative response that had been decreased with lidocaine did not recover with the addition of rIL-2 to the culture medium, a finding that suggests that T cells were not rendered anergic (data not shown). Lidocaine inhibit-

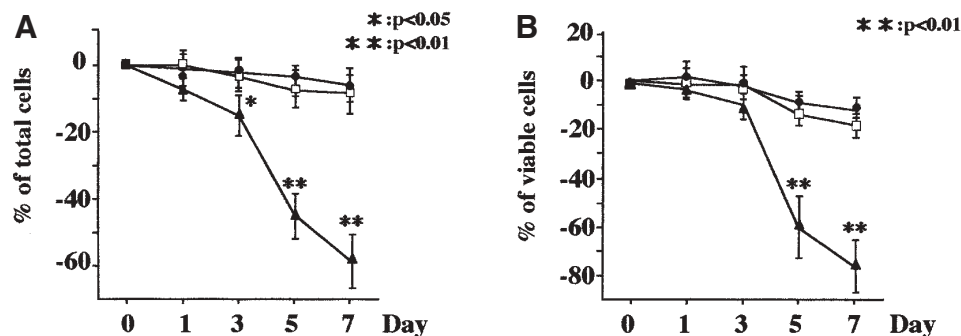


FIG 1. Changes in viability of CD4⁺ T cells after incubation with lidocaine. CD4⁺ T cells from 6 patients with allergic asthma were cultured for up to 7 days in the presence or absence of lidocaine (circles, 0 μmol/L lidocaine; squares, 100 μmol/L lidocaine; triangles, 1000 μmol/L lidocaine). The percentage of total cells (A) and the percentage of viable cells (B) were calculated. Total cell number and cell viability decreased significantly with 1000 μmol/L lidocaine.

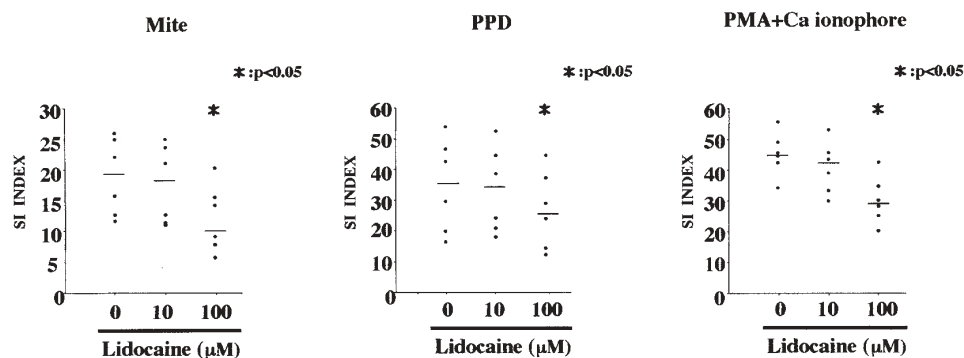


FIG 2. Proliferative responses of PBMCs from 6 patients with allergic asthma after stimulation with *D. farinae*, PPD, or PMA plus calcium ionophore in the presence or absence of lidocaine. The stimulation index (SI) was calculated. Proliferative responses were significantly decreased with 100 μmol/L lidocaine.

ed proliferative response induced by activation with PMA plus calcium ionophore in a similar way when PBMCs from healthy donors were used (stimulation index: 41.45 ± 8.4 at 0 μmol/L to 30.10 ± 7.6 at 100 μmol/L, $P < .05$).

Cytokine production

Stimulation with *D. farinae* or PPD allergens increased mRNA expression of IL-4 and IL-5 or IFN- γ in PBMCs. Addition of lidocaine inhibited the mRNA expression of these cytokines in a dose-dependent manner ($P < .05$, Fig 3). The inhibitory effect of lidocaine on protein production was also assessed. Addition of lidocaine again significantly inhibited both IL-5 and IFN- γ production by PBMCs in a dose-dependent manner ($P < .05$, Fig 4). Similar inhibitory effects of lidocaine on IL-5 and IFN- γ mRNA and protein production by stimulation with PMA plus calcium ionophore were also observed (data not shown). These results suggest that lidocaine inhibits cytokine production by T cells through allergen-specific and nonspecific activation.

Decrease in the inhibition on steroid-insensitive T cells by lidocaine

Because lidocaine could inhibit proliferative responses and cytokine production of T cells from patients with allergic asthma, we next studied the effect of lidocaine on steroid-insensitive T cells induced by means of preincubation of PBMCs with both IL-2 and IL-4. PBMCs from healthy donors were precultured with IL-2 plus IL-4, after which the inhibitory effects of dexamethasone or lidocaine on proliferative responses to stimulation with PMA plus calcium ionophore were assessed. As has been demonstrated, the proliferative response of T cells precultured with IL-2 plus IL-4 was not inhibited by dexamethasone (Fig 5, A). The inhibitory effect of lidocaine on the proliferative response of T cells precultured with IL-2 and IL-4 was also decreased (Fig 5, B). These results suggest that lidocaine has an inhibitory effect on steroid-sensitive T cells but not on steroid-insensitive T cells.

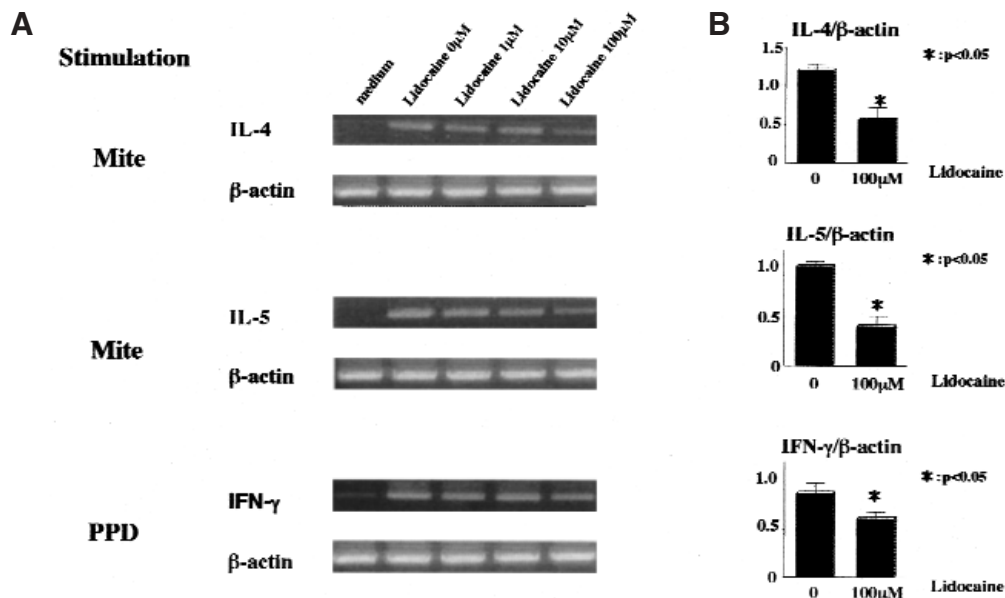


FIG 3. mRNA expression of IL-4, IL-5, and IFN- γ by PBMCs from 6 patients with allergic asthma after stimulation with *D farinae*, PPD, or PMA plus calcium ionophore in the absence or presence of lidocaine. **A**, The PCR products were electrophoresed through a 2% agarose gel and stained with ethidium bromide. **B**, Densitometric analysis of results of RT-PCR is shown, with the intensity of the cytokine signal normalized against the β -actin signal. Expression of mRNA of IL-4, IL-5, and IFN- γ was significantly decreased with 100 μ mol/L lidocaine.

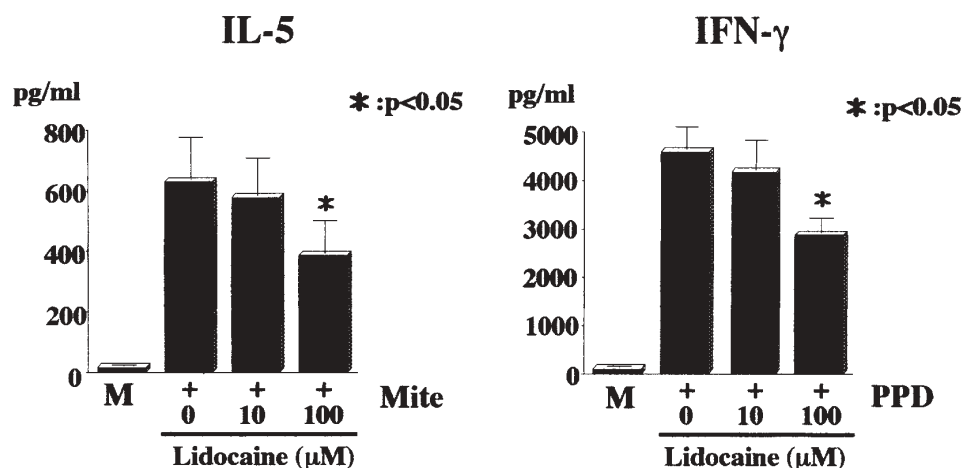


FIG 4. IL-5 and IFN- γ production by PBMCs from 6 patients with allergic asthma after stimulation with *D farinae*, PPD, and PMA plus calcium ionophore in the presence or absence of lidocaine. Cytokines were measured with ELISA. Production of IL-5 and IFN- γ was significantly decreased with 100 μ mol/L lidocaine.

DISCUSSION

Persistent airway inflammation is often found in patients with severe asthma, who often require oral corticosteroids.¹⁵ Furthermore, analysis of bronchoalveolar lavage fluid indicates that T_H2 cells are activated and that the T_H2-type cytokines, IL-4 and IL-5, are produced despite treatment with oral corticosteroids in steroid-insensitive asthma.¹⁶ Therefore, anti-inflammatory

agents other than corticosteroids are necessary to treat patients with such severe asthma. Recent studies have demonstrated that nebulized lidocaine enables oral corticosteroids to be tapered.^{9,10} Although lidocaine is reported to have anti-inflammatory effects, its effects on T cells have not been fully investigated. In the present study, we found that lidocaine inhibits the proliferation of and cytokine production by T cells through a mechanism other than apoptosis. Therefore, these results suggest that

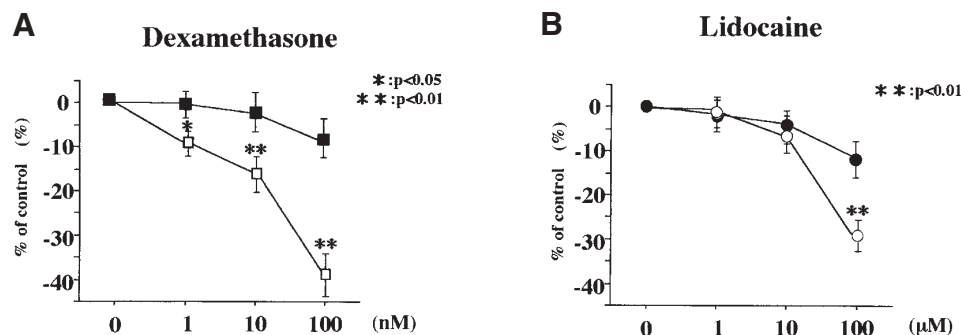


FIG 5. Proliferative responses of steroid-insensitive PBMCs from 6 nonallergic donors induced by IL-2 plus IL-4 after stimulation with PMA plus calcium ionophore in the presence of dexamethasone (A) or lidocaine (B). Horizontal bars indicate the percentage changes in proliferative response compared with control values. Proliferative responses of cells stimulated with PMA plus calcium ionophore in the presence of dexamethasone or lidocaine immediately after purification were investigated as a control. Open squares, Control PBMCs in the presence of dexamethasone; filled squares, steroid-insensitive PBMCs in the presence of dexamethasone; open circles, control PBMCs in the presence of lidocaine; filled circles, steroid-insensitive PBMCs in the presence of lidocaine. Preincubation of PBMCs with IL-2 and IL-4 significantly decreased the inhibitory effects of both dexamethasone and lidocaine.

lidocaine might be studied as an alternative to corticosteroids to treat severe steroid-dependent asthma.

Previous studies have demonstrated that lidocaine has immunoregulatory effects on mast cells, eosinophils, and neutrophils.^{11-13,17} Histamine release from mast cells is also decreased in a dose-dependent manner by means of incubation with lidocaine by inhibiting an increase in intracellular calcium- and sodium-channel transport.¹¹ Incubation with lidocaine can also induce eosinophil apoptosis in vitro.^{12,13} Lidocaine inhibits the generation of oxygen derivatives from neutrophils and also inhibits an increase in the expression of CD11b on neutrophils.¹⁸ Here we have demonstrated that lidocaine inhibits proliferative responses of T cells to specific and nonspecific stimulation. In addition, both T_H1 - and T_H2 -type cytokine production was inhibited by lidocaine in a dose-dependent manner.

The inhibitory effects of lidocaine on T cells are not likely to be due to apoptosis or necrosis because the viability of $CD4^+$ T cells was unchanged after incubation with 100 $\mu\text{mol/L}$ lidocaine. That mRNA expression and production of IL-4, IL-5, and IFN- γ were inhibited by lidocaine after stimulation with PMA plus calcium ionophore suggests that lidocaine directly inhibits T cells. In other experiments, we have studied the effects of lidocaine (1 mmol/L) on normal human fibroblasts and found that proliferative responses and extracellular matrix production are not inhibited (unpublished observations). These results suggest that lidocaine specifically affects T cells in vitro. Moreover, T cells are more sensitive to lidocaine because the concentration of lidocaine that significantly suppressed the function of T cells is one tenth that necessary to induce apoptosis of eosinophils or to inhibit histamine release from mast cells.

In a preliminary study, lidocaine was detected at concentrations of 1.7 to 10.7 mmol/L in patients with asthma

who underwent safety bronchoscopy.¹² Although no study has measured the concentration of lidocaine in the airway after inhalation of nebulized lidocaine, the clinical efficacy of nebulized lidocaine therapy that enabled dosages of oral corticosteroids to be tapered in severe asthma strongly suggests that lidocaine might inhibit T cells. Therefore, the lidocaine concentration of 100 $\mu\text{mol/L}$ used in the present study might be achieved in vivo.

Lidocaine is widely used as a short-acting local anesthetic and as an antiarrhythmic agent because of its function in Na^+ and Ca^{2+} channel blockade.¹⁹ Previous studies suggest that lidocaine suppresses histamine release by inhibiting Ca^{2+} influx in mast cells. In addition, lidocaine directly relaxes airway smooth muscle by decreasing intracellular Ca^{2+} .²⁰ Activation of several ionic channels, including Ca^{2+} , K^+ , and Cl^- channels, is important in the early phase of T-cell activation.^{21,22} Elevation of Ca^{2+} plays a critical role in the activation and translocation of nuclear transcription factors, including nuclear factor AT, nuclear factor κB , and JNK.^{23,24} Moreover, a recent study suggests that activation of voltage-sensitive and voltage-gated Na^+ channels is an important step in allowing an adequate influx of Na^+ and maintaining a sustained high Ca^{2+} level during human T-cell activation.²⁵ Therefore, the inhibitory effects of lidocaine on proliferative response and cytokine production may be partly induced by the inhibition of Ca^{2+} and Na^+ channels. Further studies are necessary to elucidate the effects of lidocaine on ion channels in T_H1 and T_H2 cell function.

Previous studies have shown that corticosteroids do not inhibit proliferative response and cytokine production by T cells after stimulation with mitogen in steroid-insensitive asthma.²⁶ In addition, T cells from patients with steroid-insensitive asthma are persistently activated, despite treatment with oral corticosteroids.¹⁶ Because lidocaine has inhibitory effects on T cells, we next stud-

ied the effect of lidocaine on steroid-insensitive T cells. In vitro incubation of normal peripheral blood T cells with IL-2 plus IL-4 reduces their glucocorticosteroid receptor ligand-binding affinity to the level seen in steroid-insensitive asthma.¹⁴ Therefore, we used these T cells as a model of steroid-insensitive T cells. In the present study, lidocaine failed to inhibit the proliferative response of steroid-insensitive T cells after stimulation with PMA plus calcium ionophore. Although the precise reason for this finding is unknown, our data suggest that nebulized lidocaine may not be an effective alternative to oral corticosteroid therapy in patients with steroid-insensitive asthma.

In conclusion, we have demonstrated that lidocaine has immunoregulatory effects on T cells. Therefore, lidocaine might be studied as an anti-inflammatory agent in the treatment of severe steroid-dependent asthma.

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