

Enhancement of in vitro spontaneous IgE production by topical steroids in patients with atopic dermatitis

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Background: Atopic dermatitis (AD) is an inflammatory skin disease. Although topical steroids are widely used for AD, management of severe AD is not satisfactory because of relapse or occasional aggravation of symptoms. Moreover, glucocorticoids induce in vitro IgE production. On the other hand, topical sodium cromoglycate (SCG) solution is a safe and effective treatment for AD.

Methods: We treated 43 patients with AD with SCG solution ($n = 21$) or with topical steroids, beclomethasone dipropionate (BD) ointment ($n = 22$). After 2 weeks, clinical evaluation and spontaneous immunoglobulin production by peripheral blood B cells or surface IgE⁺ B cells from patients in the SCG and BD groups were assessed.

Results: Both SCG and BD treatment remarkably improved eczema. However, although SCG treatment decreased spontaneous IgE production by B cells without affecting production of IgG, IgM, or IgA, BD treatment selectively increased spontaneous IgE production. SCG treatment also decreased IgE production by surface IgE⁺ B cells, whereas BD treatment increased it.

Conclusion: Topical steroid treatment increases in vitro spontaneous IgE production by B cells. This indicates that topical steroids may decrease inflammation; however, a large-scale study on the effect of topical steroids on IgE production in vitro and in vivo may be necessary. (*J Allergy Clin Immunol* 1996;98:107-13.)

Key words: IgE production, topical steroid, eczema, atopic dermatitis, IL-4, sodium cromoglycate

Atopic dermatitis (AD) is an inflammatory skin disease of wide prevalence characterized by eczema, chronic pruritus, and increased serum IgE. Although topical steroids are widely used, the disease is often associated with relapse after therapy has been discontinued.^{1,2} On the other hand, we and others have reported that glucocorticoids induce IgE production if they are added to in vitro culture.³⁻⁵ We have also previously reported that topical sodium cromoglycate (SCG) treatment is safe and effective for AD.^{2,6,7} Such treatment also decreases in vitro

Abbreviations used

AD:	Atopic dermatitis
BD:	Beclomethasone dipropionate
BSA:	Bovine serum albumin
FACS:	Fluorescence-activated cell sorter
IFN:	Interferon
mAB:	Monoclonal antibody
PBS:	Phosphate-buffered saline
SCG:	Sodium cromoglycate
sIg ⁺ :	Surface immunoglobulin positive
TNF:	Tumor necrosis factor

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spontaneous IgE production by peripheral blood mononuclear cells.^{6,7} Moreover, SCG also selectively inhibits IgE production when added to in vitro culture.⁸ These results prompted us to attempt to determine whether topical steroid treatment in patients with AD affects in vitro spontaneous IgE production by B cells.

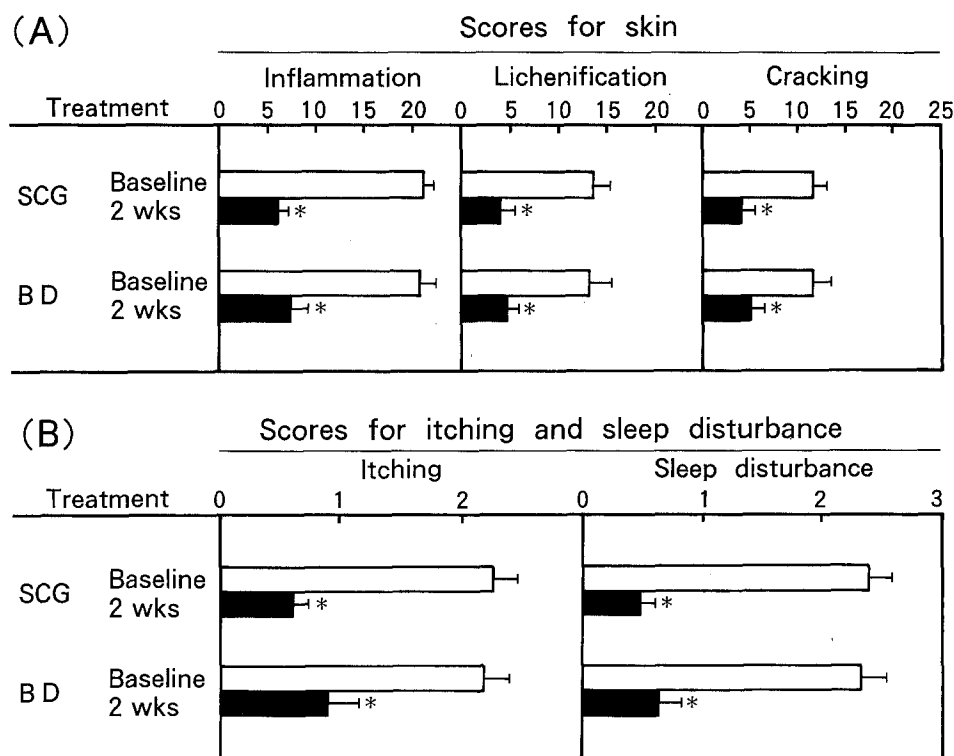


FIG. 1. Scores for skin, itching, and sleep disturbance. Patients were treated with SCG ($n = 21$) or BD ($n = 22$) for 2 weeks, and scores for skin (A) and itching and sleep disturbance (B) were determined. Results are expressed as means $\pm$ SEM. Asterisk indicates significant improvement ($p < 0.01$) compared with baseline in each group.

METHODS

Patients

Forty-three patients, (22 boys and 21 girls, 5.2 to 14.6 years old) were enrolled in this study. Informed consent was obtained from patients' parents. All patients had moderate to severe AD, according to the standard grading system.^{1,2} All had positive RAST scores for house dust and mite. The grade of AD was assessed by using our scoring system.^{2,6,7} Grading was done on a scale of 0 to 2 in ascending order of severity with respect to inflammation, lichenification, and cracking. These signs were assessed on 15 areas of the body (face, scalp, neck, right arm, left arm, right hand, left hand, trunk, back, pelvis, buttocks, right leg, left leg, right foot, and left foot). The maximum possible score was 30. Diary card scores for itching and sleep disturbance were graded on a scale of 0 to 3 in ascending order of severity, as previously reported.^{2,6,7}

All patients stopped taking medication, including oral antiallergic medication and nonsteroid ointments, 2 days before the study. None had taken oral antihistamines. The study was designed as a double-blind, randomized-group, comparative trial. By random selection, one group of patients ($n = 21$; 11 boys and 10 girls, 5.2 to 14.2 years old) was treated with topical SCG solution (Fisons plc, Loughborough, U.K.): SCG nebulizer solution was simply applied to the affected skin three times

daily. The other group ($n = 22$; 12 boys and 10 girls, 5.4 to 14.6 years old) was treated with simple application of beclomethasone dipropionate (BD) ointment three times daily. No other medication, including oral or topical antihistamines and antiallergic drugs, was prescribed. There was no difference between the two groups with regard to severity of AD (Fig. 1), age, and serum IgE levels as determined by RAST (Table I). At baseline (before treatment) and after 2 weeks, scores for skin, itching, and sleep disturbance were assessed.^{6,7} Compliance with application of SCG and BD was confirmed by doctors during a follow-up visit to the hospital.

Cell culture

Peripheral blood was obtained from patients on day 0 (baseline) and after 2 weeks of SCG or BD treatment. Mononuclear cells were obtained by means of buoyant density centrifugation on a Ficoll-Hypaque gradient (Pharmacia, Uppsala, Sweden). T cells were separated from mononuclear cells by sheep red blood cell rosetting, and monocytes and natural killer cells in T cells were depleted by using the L-leucine methyl ester preincubation method.^{9,10} The T-cell fractions contained less than 1% CD20⁺ B cells, less than 1% CD14⁺ monocytes, less than 1% CD16⁺ natural killer cells, and more than 98% CD3⁺ T cells as determined by fluorescence-activated cell sorter (FACS). On the other hand,

T cell-depleted B-cell fractions were further depleted of monocytes and natural killer cells by using the L-leucine methyl ester preincubation method.^{8,9} The purified B-cell fractions contained less than 1% CD3⁺ T cells, less than 1% CD14⁺ monocytes, less than 1% CD16⁺ natural killer cells, and more than 98% CD20⁺ B cells as determined by FACS. In some experiments the percentage of surface (s)IgE⁺ B cells before and after SCG or BD treatment was determined by FACS. On the other hand, sIgE⁺ B cells and other sIg⁺ B cells were purified by panning, as previously reported, by using mouse IgG₁ anti-IgE monoclonal antibody (mAb) (7.12, kindly provided by Dr. Andrew Saxon, University of California Los Angeles), mouse IgG₁ anti-IgG mAb, mouse IgG₁ anti-IgM mAb, and mouse IgG₁ anti-IgA mAb (Cosmo Bio. Co., Ltd., Tokyo, Japan).^{9,10} Each purified sIg⁺ B-cell fraction contained more than 98% of corresponding sIg⁺ B cells as determined by FACS. B cells (1×10^4 cells/0.2 ml/well) or sIg⁺ B cells (1×10^4 cells/0.2 ml/well) were cultured in 96-well U-bottom microtiter plates (Costar, Cambridge, Mass.) with RPMI-1640 medium (M.A. Bioproduct, Walkersville, Md.) containing 10% fetal calf serum (Irvine Scientific, Santa Ana, Calif.), 50 mg/ml transferrin,¹¹ 2 mmol/L L-glutamine, 50 U/ml penicillin, and 50 mg/ml streptomycin in a humidified incubator containing 5% CO₂ in air at 37°C.^{9,12,13} We previously found that spontaneous immunoglobulin production in atopic patients reached a plateau after 10 days of culture under these conditions and that purified B cells and sIgE⁺ B cells spontaneously produced IgE up to 25 ng/ml and 40 ng/ml, respectively.⁵ It has been reported that steroid-induced immunoglobulin production in B cells from healthy donors is dependent on contact of B cells with monocytes for 14 days of culture.¹⁴ In contrast, purified B cells or sIgE⁺ B cells from atopic patients contained in vivo activated B cells, which produced IgE spontaneously and survived for 8 to 10 days (10% to 20% viability), as we and others have previously reported.^{5,15} Therefore immunoglobulin production was measured after 10 days of culture by ELISA (see below).^{9,12,16} In some experiments the binding of tumor necrosis factor (TNF)- α and IL-6 to sIgE⁺ B cells was studied by using biotinylated TNF- α and IL-6 (R & D Systems, Minneapolis, Minn.) as previously reported.¹⁷ Briefly, sIgE⁺ B cells were cultured (1×10^5 cells/0.2 ml/well) with medium for 2 days, washed with acidic buffer, and incubated for 3 hours at 4°C in the presence of 5 nmol/L biotinylated TNF- α or IL-6; they were then stained with streptavidin-phycoerythrin and analyzed with a FACScan (Becton-Dickinson, Mountain View, Calif.). The mean fluorescence intensity (MFI) value of biotinylated cytokine-specific binding, determined after the subtraction of nonspecific binding in the presence of a 100-fold excess of unlabeled cytokines, was expressed as Δ MFI.¹⁷ Surface IgE⁺ B cells were also cultured (1×10^4 cells/0.2 ml/well) with medium for 2 days, and the production of TNF- α and IL-6 in sIgE⁺ B cells was determined by ELISA (R & D Systems).¹⁷ In

TABLE I. Effect of SCG or BD treatment on percentages of sIgE⁺ B cells and serum IgE levels

Treatment	Percentage of sIgE ⁺ B cells*	Serum IgE (IU/ml)†
SCG		
Baseline	15.3 $\pm$ 2.4	3212 $\pm$ 428
2 weeks	16.7 $\pm$ 2.1	3101 $\pm$ 392
BD		
Baseline	14.8 $\pm$ 2.8	3146 $\pm$ 501
2 weeks	15.2 $\pm$ 3.0	3026 $\pm$ 437

*Mean $\pm$ SEM of percentage of sIgE⁺ B cells in B-cell fraction on day 0 (baseline) and after 2 weeks of treatment ($n = 21$).

†Mean $\pm$ SEM of serum IgE levels on day 0 (baseline) and after 2 weeks of treatment ($n = 22$).

some experiments T cells (1×10^5 cells/0.2 ml/well) were cultured with medium or 1 ng/ml phorbol myristate acetate (Sigma Chemical Co., St. Louis, Mo.) plus 0.1 mg/ml phytohemagglutinin (Wellcome Laboratories, Beckenham, U.K.) for 2 days, and IL-4 and interferon (IFN)- γ production were measured by ELISA (R & D Systems).¹⁷

Immunoglobulin measurement by ELISA

Immunoglobulin from culture supernatants was measured by isotype-specific ELISA. Flat-bottomed microtiter plates (Dynatech Laboratories, Inc., Alexander, Va.) were coated with mixtures of mouse anti-IgE mAb (2 mg/ml) (7.12 and 4.15, kindly provided by Dr. Andrew Saxon), anti-IgG antibody (200 mg/ml; Tago Inc., Burlingame, Calif.), anti-IgM antibody (200 mg/ml, Tago), and anti-IgA antibody (200 mg/ml, Tago). The plates were incubated for 1 hour at room temperature and washed three times with phosphate-buffered saline (PBS)–0.05% Tween-20 (Sigma Chemical Co.) plus 0.01% thimerosal (Sigma) (PBS-E), then blocked with 1% bovine serum albumin (BSA)–PBS for 30 minutes. After draining the plates, 10 to 25 ml of supernatants and standard curves of the appropriate purified immunoglobulin were added, and 1% BSA-PBS was added to a final volume of 100 ml/well. After incubation for 2 hours at 23°C and washing four times with PBS-E, affinity-purified isotype-specific peroxidase-conjugated goat anti-human immunoglobulin diluted in 1% BSA-PBS was added for 1 hour. The developing antibodies for IgE, IgG, IgM, and IgA were diluted at 1:4000 (Kirkgaard & Perry Laboratories, Gaithersburg, Md.). Thereafter, the plates were washed four times with PBS-E and allowed to react with 100 ml of 2.2 mmol/L O-phenylenediamine (Eastman Kodak Co., Rochester, N.Y.) in 0.1 mol/L citrate solution, pH 5.0, containing 0.012% H₂O₂. The reaction was terminated with 100 ml of 1.8 mol/L H₂SO₄. Color development was measured by using an Autoreader (model ELK 310; Bio-Tek

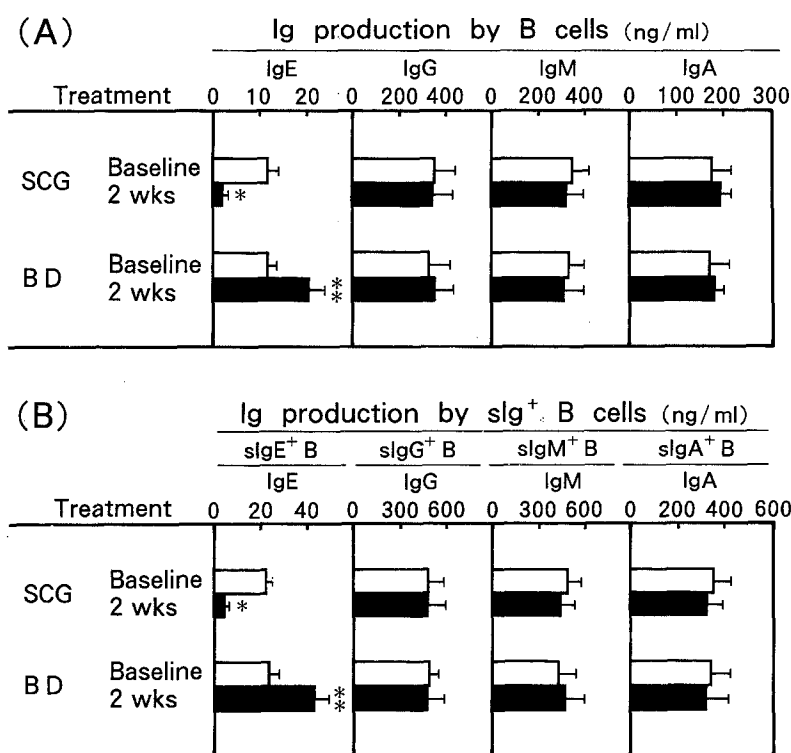


FIG. 2. Spontaneous immunoglobulin production by B cells and sIg⁺ B cells. Purified B cells **(A)** and sIg⁺ (sIgE⁺, sIgG⁺, sIgM⁺, and sIgA⁺) B cells **(B)** from patients treated with SCG ($n = 21$) or BD ($n = 22$) were cultured with medium. After 10 days of culture, immunoglobulin production was determined. Results are expressed as means $\pm$ SEM. Asterisk indicates significant decrease ($p < 0.01$). Two asterisks indicate significant increase ($p < 0.01$) compared with baseline in each group.

Instruments, Inc., Winooski, Vt.). Samples were run in triplicate. The sensitivity of the assays was 0.2 ng/ml for IgE and 0.6 ng/ml for IgG, IgM, and IgA. The specificity of the assay was confirmed as reported previously.^{5,18} Levels of IgE as low as 0.2 ng/ml and levels of IgG, IgM, and IgA as low as 0.6 ng/ml could be detected in the presence of other immunoglobulin at a concentration of 2 mg/ml. However, we found that some lots of BSA cross-reacted with peroxidase-conjugated anti-human immunoglobulin. Therefore BSA was screened before assay, and non-cross-reactive BSA was used. Intra- and interassay variability were less than 15%, as reported previously.¹⁹ Statistical analysis was done by Student's t test.

RESULTS

Effects of SCG and BD on AD

After 2 weeks of treatment, eczema improved significantly as assessed by skin scores for inflammation, lichenification, and cracking in both the SCG and BD groups (Fig. 1, A). Itching and sleep disturbance also decreased significantly in the two groups (Fig. 1, B). There was no difference in efficacy of treatment between the two groups.

Effects of SCG and BD on immunoglobulin production

As shown in Fig. 2, A, SCG treatment significantly decreased in vitro IgE production without affecting production of IgG, IgM, or IgA. In contrast, BD treatment selectively increased IgE production (Fig. 2, A). We have previously reported that sIgE⁺, but not sIgE⁻, B cells from patients with AD produced IgE spontaneously in vitro.⁹ As shown in Fig. 2, B, IgE production in sIgE⁺ B cells was decreased in the SCG group, but it was enhanced in the BD group. Although not shown in either group, sIgE⁻ B cells failed to produce IgE (<0.2 ng/ml). In contrast, IgG, IgM, and IgA production in sIgG⁺, sIgM⁺, and sIgA⁺ B cells, respectively, was not changed by either SCG or BD treatment. However, the percentage of sIgE⁺ B cells in the B-cell fraction at baseline and after 2 weeks of treatment was not changed in either the SCG group or the BD group (Table I). Moreover, serum IgE levels were not changed by SCG or BD treatment (Table I).

TABLE II. Effects of SCG or BD treatment on IL-4 and IFN- γ production by T cells

Treatment	Cytokine production (pg/ml)*			
	Spontaneous*		PMA plus PHA†	
	IL-4	IFN- γ	IL-4	IFN- γ
SCG				
Baseline	327 $\pm$ 23	47 $\pm$ 5	1546 $\pm$ 168	1834 $\pm$ 173
2 weeks	293 $\pm$ 34	50 $\pm$ 7	1621 $\pm$ 243	1712 $\pm$ 101
BD				
Baseline	318 $\pm$ 26	42 $\pm$ 6	1575 $\pm$ 145	1792 $\pm$ 205
2 weeks	289 $\pm$ 24	38 $\pm$ 7	1492 $\pm$ 191	1767 $\pm$ 165

PMA, Phorbol myristate acetate; PHA, phytohemagglutinin.

*Mean $\pm$ SEM of spontaneous IL-4 and IFN- γ production by T cells on day 0 (baseline) and after 2 weeks of treatment ($n = 21$).

†Mean $\pm$ SEM of PMA plus PHA-induced IL-4 and IFN- γ production by T cells on day 0 (baseline) and after 2 weeks of treatment ($n = 22$).

Effect of SCG and BD on production of cytokines by T cells

IgE production was induced by IL-4, but it was inhibited by IFN- γ .⁸ Therefore production of these cytokines by T cells was studied in the SCG and BD groups. As shown in Table II, neither spontaneous production of IL-4 and IFN- γ nor that induced by phorbol myristate acetate plus phytohemagglutinin was modulated by SCG or BD treatment.

Mechanisms of modulation of IgE production by SCG and BD

We have previously reported that spontaneous IgE production in atopic patients' B cells was enhanced by TNF- α and IL-6, whereas it was not modulated by IL-4 or IFN- γ .^{17, 20} It is thus possible that modulation of spontaneous IgE production is due to a decrease or increase in endogenous production of TNF- α and/or IL-6 by sIgE⁺ B cells, or alternatively, it is due to change of expression of receptors for IL-6 and/or TNF- α on sIgE⁺ B cells. To clarify this, sIgE⁺ B cells were cultured with medium, and production and binding of TNF- α and IL-6 were determined. As shown in Table III, production of TNF- α and IL-6 in sIgE⁺ B cells was decreased in the SCG group, whereas the production of those cytokines was not changed in the BD group. Moreover, binding of TNF- α and IL-6 on sIgE⁺ B cells was decreased; however, in contrast, binding of these cytokines was increased in the BD group (Table III).

TABLE III. Effect of SCG or BD treatment on production and binding of TNF- α and IL-6 in sIgE⁺ B cells

Treatment	Production (pg/ml)*		Binding (Δ MFI)†	
	TNF- α	IL-6	TNF- α	IL-6
SCG				
Baseline	178 $\pm$ 18	161 $\pm$ 14	42 $\pm$ 4	52 $\pm$ 3
2 weeks	75 $\pm$ 4‡	62 $\pm$ 5‡	19 $\pm$ 2‡	24 $\pm$ 3‡
BD				
Baseline	158 $\pm$ 17	149 $\pm$ 13	40 $\pm$ 5	49 $\pm$ 6
2 weeks	142 $\pm$ 14	135 $\pm$ 10	64 $\pm$ 7§	69 $\pm$ 8§

MFI, Mean fluorescence intensity.

*Mean $\pm$ SEM of spontaneous TNF- α and IL-6 production on day 0 (baseline) and after 2 weeks of treatment ($n = 21$).

†Mean $\pm$ SEM of binding of TNF- α and IL-6 on day 0 (baseline) and after 2 weeks of treatment ($n = 22$).

‡Significant decrease compared with baseline ($p < 0.01$).

§Significant increase compared with baseline ($p < 0.01$).

DISCUSSION

We have demonstrated that although both SCG and BD treatment improve AD, their effects on in vitro IgE production are different. Although SCG treatment decreased IgE production without affecting production of IgG, IgM, and IgA, BD treatment selectively increased IgE production. Moreover, in the SCG group IgE production by sIgE⁺ B cells was enhanced; but production of IgG, IgM, and IgA by sIgG⁺, sIgM⁺, and sIgA⁺ B cells, respectively, was not affected. In contrast, in the BD group IgE production by sIgE⁺ B cells was selectively decreased. These results indicate that sIgE⁺ B cells were specifically activated by SCG treatment, whereas they were inhibited by BD treatment.

Glucocorticoids have been shown to induce IgE production in purified B cells.³⁻⁵ On the other hand, we and others have reported that IL-4 production by T cells or mononuclear cells is enhanced, IFN- γ production is decreased in patients with AD, and sIgE⁺ B cells are activated by IL-4 in vivo and produce IgE spontaneously.^{8, 17, 21} Our subsequent study has revealed that IL-4 or IFN- γ production by T cells was not changed by treatment with SCG or BD. In addition, the percentage of sIgE⁺ B cells was not changed in either group after treatment. We have also reported that endogenous TNF- α and IL-6, but not IL-4 or IL-13, are necessary for spontaneous IgE production.^{17, 20} Our results indicated that production of TNF- α and IL-6 by IgE⁺ B cells was decreased by SCG treatment, whereas production was not

changed by BD treatment. Moreover, expression of receptors for TNF- α and IL-6 was downregulated by SCG treatment, whereas expression of those receptors was upregulated by BD treatment. This is unexpected but not surprising, because SCG downregulated expression of complement receptor and Fc γ receptor, whereas glucocorticoid upregulated IL-6 receptor expression.^{22, 23} Collectively, these results indicate that mechanisms of decreased IgE production caused by SCG treatment may be due to decreased production of TNF- α and IL-6 and downregulation of expression of receptors for TNF- α and IL-6 in sIgE⁺ B cells. In contrast, increased IgE production may be due to upregulation of expression of receptors for TNF- α and IL-6 in sIgE⁺ B cells.

There are two possible mechanisms for this. First, it is possible that SCG or BD directly affects sIgE⁺ B cells by absorption through skin. However, this is unlikely because SCG was not detectable in the plasma by radioimmunoassay after treatment ($n = 16$). Moreover, plasma BD after BD treatment was not detectable ($n = 16$). Second, SCG or BD treatment induces factor(s), which in turn affect sIgE⁺ B cells. It has been reported that skin keratinocytes, fibroblasts, T cells, and B cells produce cytokines, including IL-6, IL-8, and TNF- α , which modulate IgE production.²⁴⁻²⁶ Moreover, plasma IL-8 and TNF- α levels were elevated in patients with AD.^{7, 27} It is thus possible that production of these cytokines is modulated by SCG or BD, which in turn affects sIgE⁺ B cells and modulates IgE production. Indeed, we found that plasma TNF- α concentrations were decreased after treatment in the SCG group, but not in the BD group; plasma TNF- α concentrations in the SCG group at baseline and after 2 weeks of treatment were 79 ± 7 pg/ml and 21 ± 3 pg/ml, respectively. In contrast, plasma TNF- α concentrations in the BD group at baseline and after 2 weeks were 73 ± 6 pg/ml and 68 ± 5 pg/ml, respectively. The lack of effect of BD treatment on TNF- α production is not surprising, because even in vivo administration of hydrocortisone has no effect on serum TNF- α levels.²⁸ We are currently studying the detailed mechanisms of this cytokine and also measuring plasma concentrations of other cytokines.

Despite the change of in vitro IgE production by sIgE⁺ B cells, serum IgE levels were not changed by SCG or BD treatment. The mechanism of this phenomenon is currently under investigation. However, we previously reported that topical SCG treatment in AD decreased in

vitro IgE production by mononuclear cells without decreasing serum IgE levels.⁶ Treatment of asthmatic patients with inhaled SCG decreased IgE levels in the bronchoalveolar lavage fluid, but not in the serum.²⁹ Treatment of patients with AD by IFN- γ decreased in vitro IgE production by mononuclear cells, but serum IgE levels were not decreased.³⁰ It is possible that long-term treatment may be required to reduce ongoing in vivo IgE production, whereas short-term treatment reduces in vitro IgE production. Indeed, we found that longer treatment with SCG decreased serum IgE levels: mean serum IgE levels in the SCG group ($n = 21$) were 2513 IU/ml and 1954 IU/ml, after 6 and 8 weeks, respectively. Whether other factors, such as reduction of plasma IL-6 levels, may be necessary is currently under investigation. Taken together, these results suggest that change of in vitro IgE production may precede that of serum IgE levels, and measurement of in vitro IgE production may be useful for studying the clinical course of AD.

In conclusion, although steroid ointment treatment may be effective in AD, it increases in vitro IgE production. It may be necessary to study the detailed analysis of longer treatment of steroid ointment on IgE production in vitro and in vivo on a large scale.

REFERENCES

1. Rajika G, Langeland T. Grading the severity of atopic dermatitis. *Acta Derm Venereol Suppl* (Stockh) 1989;144:13-4.
2. Kimata H, Igarashi M. Topical cromolyn (disodium cromoglycate) solution in the treatment of young children with atopic dermatitis. *Clin Exp Allergy* 1990;20:281-3.
3. Jabara HH, Ahren DJ, Vercelli D, Geha RS. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. *J Immunol* 1991;147:1557-60.
4. Wu CY, Sarfati M, Heusser C, Fournier S, Rubio-Trujillo M, Delespesse G. Glucocorticoids increase the synthesis of immunoglobulin E by interleukin 4-stimulated human lymphocytes. *J Clin Invest* 1991;87:870-7.
5. Kimata H, Lindley I, Furusho K. Effect of hydrocortisone on spontaneous IgE and IgG4 production in atopic patients. *J Immunol* 1995;154:3557-66.
6. Kimata H, Hiratsuka S. Effect of topical cromoglycate solution on atopic dermatitis: combined treatment of sodium cromoglycate solution with the oral anti-allergic medication, oxatomide. *Eur J Pediatr* 1994;153:66-71.
7. Kimata H, Lindley I. Detection of plasma interleukin-8 in atopic dermatitis. *Arch Dis Child* 1994;70:119-22.
8. Kimata H, Yoshida A, Ishioka C, Mikawa H. Disodium cromoglycate (DSCG) selectively inhibits IgE production and enhances IgG4 production by human B cells in vitro. *Clin Exp Immunol* 1991;84:395-9.
9. Kimata H, Mikawa H. Nedocromil sodium selectively inhibits IgE and IgG4 production in human B cells stimulated with IL-4. *J Immunol* 1993;151:6723-32.

10. Kimata H, Saxon A. Subset of natural killer cells is induced by immune complexes to display Fc receptors for IgE and IgA and demonstrates isotype regulatory function. *J Clin Invest* 1988;82:160-7.
11. Van der Pouw-Kraan T, Koten CV, Oers RV, Aarden LA. Human transferrin allows efficient IgE production by anti-CD3-stimulated human lymphocytes at low cell densities. *Eur J Immunol* 1991;21:385-90.
12. Kimata H, Yoshida A, Ishioka C, Kusunoki T, Hosoi S, Mikawa H. Nerve growth factor specifically induces human IgG4 production. *Eur J Immunol* 1991;21:137-41.
13. Kimata H, Yoshida A, Fujimoto M, Mikawa H. Effect of vasoactive intestinal peptide, somatostatin, and substance P on spontaneous IgE and IgG4 production in atopic patients. *J Immunol* 1993;150:4630-40.
14. Fisher A, Konig W. Influence of cytokines and cellular interaction on the glucocorticoid-induced immunoglobulin (E, G, A, M) synthesis of peripheral blood mononuclear cells. *Immunology* 1991;74:228-33.
15. Renz H, Brodie C, Bradley K, Leung DYM, Gelfand EW. Enhancement of IgE production by anti-CD40 antibody in atopic dermatitis. *J Allergy Clin Immunol* 1994;93:658-68.
16. Kimata H, Shanahan F, Brogan M, Targan S, Saxon A. Modulation of ongoing human immunoglobulin synthesis by natural killer cells. *Cell Immunol* 1987;107:74-88.
17. Kimata H. Differential effects of gangliosides on human IgE and IgG4 production. *Eur J Immunol* 1995;25:302-5.
18. Kimata H. High-dose intravenous γ -globulin treatment for hyperimmunoglobulinemia E syndrome. *J Allergy Clin Immunol* 1995;95:771-4.
19. Kimata H, Yoshida A, Ishioka C, Jiang Y, Mikawa H. Eosinophil cationic protein inhibits immunoglobulin production and proliferation in vitro in human plasma cells. *Cell Immunol* 1992;141:422-32.
20. Kimata H, Fujimoto M. Growth hormone and insulin-like growth factor I induce immunoglobulin (Ig)E and IgG4 production by human B cells. *J Exp Med* 1994;180:727-32.
21. Rousset F, Robert J, Anadary M, et al. Shifts in interleukin-4 and interferon- γ production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J Allergy Clin Immunol* 1991;87:58-69.
22. Kay AB, Walsh GM, Moqbel R, et al. Disodium cromoglycate inhibits activation of human inflammatory cells in vitro. *J Allergy Clin Immunol* 1987;80:1-8.
23. Snyers L, Wit LD, Content J. Glucocorticoid up-regulation of high-affinity interleukin 6 receptors on human epithelial cells. *Proc Natl Acad Sci USA* 1990;87:2838-42.
24. Banchereau J, Rousset F. Human B lymphocytes: phenotype, proliferation, and differentiation. *Adv Immunol* 1992;52:125-262.
25. Kimata H, Yoshida A, Ishioka C, Mikawa H. Differential effect of vasoactive intestinal peptide, somatostatin, and substance P on human IgE and IgG subclass production. *Cell Immunol* 1992;144:429-42.
26. Kimata H, Yoshida A, Ishioka C, Lindley I, Mikawa H. Interleukin 8 selectively inhibits IgE production induced by interleukin 4 in human B cells. *J Exp Med* 1992;176:1227-31.
27. Sumimoto S, Kawai M, Ksajima Y, Hamamoto T. Increased plasma tumor necrosis factor- α concentration in atopic dermatitis. *Arch Dis Child* 1992;67:277-9.
28. Mark P, Kraus P, Sribante J, Havlik I, Lipman J, Johnson DW. Hydrocortisone and tumor necrosis factor in severe community-acquired pneumonia: a randomized controlled study. *Chest* 1993;104:389-92.
29. Diaz P, Galleguillos FR, Gonzalez MC, Pantin CFA, Kay AB. Bronchoalveolar lavage in asthma: the effect of disodium cromoglycate (cromolyn) on leukocyte counts, immunoglobulins, and complement. *J Allergy Clin Immunol* 1984;74:41-8.
30. Boguniewicz M, Jaffe HS, Izu A, et al. Recombinant gamma interferon in treatment of patients with atopic dermatitis and elevated IgE levels. *Am J Med* 1990;88:365-70.