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Disodium cromoglycate (DSCG) selectively inhibits IgE production and enhances IgG4 production by human B cells *in vitro*

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SUMMARY

The effect of DSCG on human IgE production *in vitro* was studied. DSCG selectively inhibited interleukin-4 (IL-4) induced IgE production by mononuclear cells (MNC) from normal donors without affecting IgM, IgA, IgG1, IgG2 or IgG3 production. In contrast, DSCG enhanced IgG4 production. To achieve this effect, DSCG must be added to the culture at the initiation and be present throughout the entire culture period. Interferon-gamma (IFN-γ) also inhibited IL-4-induced IgE production, but IgG4 production was not affected by IFN-γ. Monoclonal anti-IFN-γ antibody blocked the inhibition of IgE production by IFN-γ, but did not block the inhibition of IgE production by DSCG. DSCG also selectively inhibited spontaneous IgE production and enhanced IgG4 production by B cells from atopic patients in the presence of T cells and monocytes. These results indicate that there is a mechanism of IgE production inhibition which is not mediated by IFN-γ. We also found that DSCG is an excellent reagent for the study of IgE and IgG4 regulation *in vitro*.

Keywords disodium cromoglycate IgE and IgG4 production interleukin-4

INTRODUCTION

DSCG is well known as a prophylactic agent in the treatment of allergic disease of the respiratory tract. *In vitro*, DSCG inhibits histamine release from mast cells (Theoharides *et al.*, 1980), the activation of inflammatory cells including neutrophils, eosinophils and monocytes (Kay *et al.*, 1987), and antibody-dependent granulocyte-mediated cytotoxicity (Rand *et al.*, 1988). *In vivo*, treatment of asthmatic patients with DSCG decreased eosinophils and house-dust-mite-specific IgE in bronchoalveolar lavage (Diaz *et al.*, 1984). We have previously reported that DSCG solution was very effective in the treatment of atopic dermatitis (Kimata & Igarashi, 1990a). Moreover, DSCG inhibited human allergic skin reactions *in vivo* (Kimata & Igarashi, 1990b). These results indicate that DSCG can modulate immune responses both *in vitro* and *in vivo*. However, the effect of DSCG on IgE production *in vitro* has not been studied. *In vitro* IgE production was induced by interleukin-4 (IL-4) in cultures of mononuclear cells (MNC) from normal donors, and inhibited by interferon-gamma (IFN-γ) (Pène *et al.*, 1988). MNC from atopic patients spontaneously produced IgE which was also enhanced by IL-4 (Yang, De Weck & Stadler, 1988). IL-4 also induces IgE and IgG4 production in co-cultures of non-atopic B cells and EL4 cells in the presence of phorbol 12-myristate 13-acetate (PMA) (Lundgren *et al.*, 1989). These results indicate a possible relationship between the regulation of IgE and that of IgG4. Here we demonstrate that DSCG selectively inhibits IgE production and enhances IgG4 production in cultures of both IL-4-stimulated MNC from non-atopic donors and unstimulated MNC from atopic donors.

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MATERIALS AND METHODS

Reagents

DSCG was a gift from Fujisawa Fisons Pharmaceuticals (Osaka, Japan). DSCG was dissolved in water at 2×10^{-2} M, filtered through a Millipore filter, sterilized, and stored until use. In each experiment, DSCG was diluted in RPMI 1640 medium (M. A. Bioproducts, Walksville, MD) containing 10% fetal calf serum (FCS) (Irvine Scientific), 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. Human recombinant IL-4 and IFN-γ were kindly provided by the Ono Pharmaceutical Company (Osaka, Japan) and Takeda Chemical Industries (Osaka, Japan), respectively. Mouse monoclonal anti-human IFN-γ was purchased from Cosmo Bio Co. (Tokyo, Japan). Purified human IgG, IgM and IgA for the ELISA standard were kindly provided by Green Cross Co. (Osaka, Japan). PS IgE was kindly provided by Dr Andrew Saxon (UCLA, Los Angeles, CA).

Cells

Tonsils (kind gifts from Dr K. Nagahara, National Kyoto Hospital, Kyoto, Japan) from either non-atopic donors (serum

IgE level < 50 U/ml) or atopic patients (serum IgE level > 300 U/ml) were obtained from tonsillectomies for chronic tonsillitis. The tonsils were disrupted and MNC were obtained by buoyant density centrifugation on Ficoll–Hypaque (Kimata *et al.*, 1991). In some experiments, monocytes were obtained by plastic adherence, and T cells and B cells were separated by rosetting with sheep erythrocytes as described elsewhere (Kimata & Saxon, 1988). The contaminating monocytes and natural killer (NK) cells in the T cell fractions or B cell fractions were depleted by L-leucine methyl ester incubation methods. Briefly, cell fractions (5×10^6 cells/ml) were incubated with L-leucine methyl ester (Sigma Chemical Co., St Louis, MO) at concentrations of 5 mM at 22°C for 40 min (Thiele, Kurosaka & Lipsky, 1983). After incubation, B cell fractions contained < 1% $CD3^+$ T cells, < 1% $CD11^+$ monocytes, < 1% $CD16^+$ NK cells and > 98% $CD20^+$ B cells. T cell and monocyte fractions contained > 98% $CD3^+$ T cells and > 98% $CD11^+$ monocytes, respectively.

Cell cultures

Two kinds of cultures were set up:

IL-4-stimulated cultures of MNC from non-atopic donors. MNC (3×10^5 cells/200 μl per well) from non-atopic donors were stimulated with IL-4 (200 U/ml) in 96-well U-bottomed microtitre plates (Costar, Cambridge, MA) for 14 days in RPMI. In preliminary experiments, this concentration of IL-4 was optimized to induce maximal production of IgE, and IgE production reached a maximum on 14 days of culture in accordance with another report (Classen, Levine & Buckley, 1990). Various concentrations of DSCG, IFN-γ (100 U/ml) and/or anti-IFN-γ antibody (10 μg/ml) were added to the cultures. In the kinetic experiments, DSCG was added to IL-4-stimulated cultures on day 0 until day 5. In some experiments, MNC were pre-incubated with DSCG for 1 day, washed and then cultured with IL-4 for 14 days. Alternatively, IL-4-stimulated MNC were cultured with DSCG for various periods, and then washed and cultured with IL-4 in the absence of DSCG. After a total of 14 days of culturing, immuoglobulin production was measured.

Unstimulated cultures of MNC or B cells from atopic patients. MNC or highly purified B cells from atopic patients were cultured with various concentrations of DSCG. Purified autologous T cells and/or monocytes were added to the B cells. On the 14th day of incubation, immunoglobulin production in the culture supernatants was measured by ELISA. Net immunoglobulin production was calculated by subtracting the values of immunoglobulin obtained in parallel cultures containing cycloheximide (50 μg/ml) (Kimata, Sherr & Saxon, 1988). All experiments were done with at least two patients.

ELISA for IgM, IgA, IgE and IgG subclasses

IgM, IgA and IgE levels were measured using ELISA as described previously (Kimata & Saxon, 1988; Kimata *et al.*, 1991). IgG subclasses were measured using IgG subclass-specific mouse anti-human monoclonal antibodies as described previously (Harada *et al.*, 1989; Kimata *et al.*, 1991). Briefly, immunoplates (Nunc, Roskilde, Denmark) were coated with 1 μg/ml of mouse IgG1 anti-human IgG1 antibody (NL-16), mouse IgG1 anti-human IgG2 (HG2-56F), mouse IgG1 anti-human IgG3 (HG3-7C) or mouse IgG2b anti-human IgG4 (HG4-53G) at 4°C overnight. The immunoplates were then washed with PBS–Tween, and samples or a standard serum calibrated with a WHO reference serum pool (67/97) (kind gift from Green Cross Co.) were added and incubated for 2 h at room temperature. Peroxidase-conjugated mouse IgG2a anti-human IgG (HG-2-25) diluted to 1/1000 was then added. After 1 h of incubation at room temperature, the reaction was stopped and absorbance was measured using an ELISA reader. The sensitivities of the assays were 0·6 ng/ml for IgM, IgA, IgG1, IgG2, IgG3 and IgG4, and 0·15 ng/ml for IgE (Kimata & Saxon, 1988; Kimata *et al.*, 1991).

Table 1. Effect of IL-4 on immunoglobulin production by tonsil MNC

Experiment no.	Immunoglobulin production (ng/ml)						
	IgM	IgG1	IgG2	IgG3	IgG4	IgA	IgE
1 Medium	960	514	327	127	23	769	<0·15
IL-4	908	502	303	129	20	757	1·2
2 Medium	3201	1029	786	327	111	689	<0·15
IL-4	3019	1002	756	301	101	610	1·8

Tonsil MNC from two non-atopic donors were cultured with medium or IL-4 (200 U/ml). On day 14, immunoglobulin Ig production was measured. Values are the mean of triplicate cultures. s.d. were < 15%.

RESULTS

Effect of DSCG on immunoglobulin production in IL-4-stimulated MNC cultures

The effect of IL-4 on immunoglobulin production by MNC from normal donors was studied initially. As shown in Table 1, IL-4 induced IgE production without affecting IgM, IgA, IgG1, IgG2, IgG3 or IgG4 production. Higher concentrations of IL-4 did not enhance IgE or IgG4 production further (data not shown). Therefore, in subsequent experiments, the effect of DSCG was studied in IL-4-stimulated cultures. As shown in Fig. 1a, DSCG inhibited IgE production (80% inhibition) and enhanced IgG4 production (300–400% enhancement) in a dose-dependent fashion. In contrast, the addition of DSCG to IL-4-stimulated cultures did not affect IgM, IgA, IgG1, IgG2 or IgG3 production at any concentrations tested (Fig. 1b).

Kinetic analysis of DSCG incubation

We analysed the time-course of the DSCG effect on IgE and IgG4 production. Firstly, DSCG was added daily on days 0–5 of culture. As shown in Fig. 2, DSCG had to be added at the initiation of culturing, i.e. day 0, since DSCG did not affect IgE and IgG4 production if added after day 1. Secondly, MNC were pre-incubated with DSCG for 1 day in the absence of IL-4, and then cultured with IL-4 in the absence of DSCG. Alternatively, MNC were incubated with DSCG for various periods in the presence of IL-4, washed and then cultured with IL-4 in the absence of DSCG. As shown in Fig. 3, pre-incubation with DSCG had no effect. Moreover, DSCG had to be present during the entire 14 days of culturing for IgE production inhibition and IgG4 production enhancement to be maximized.

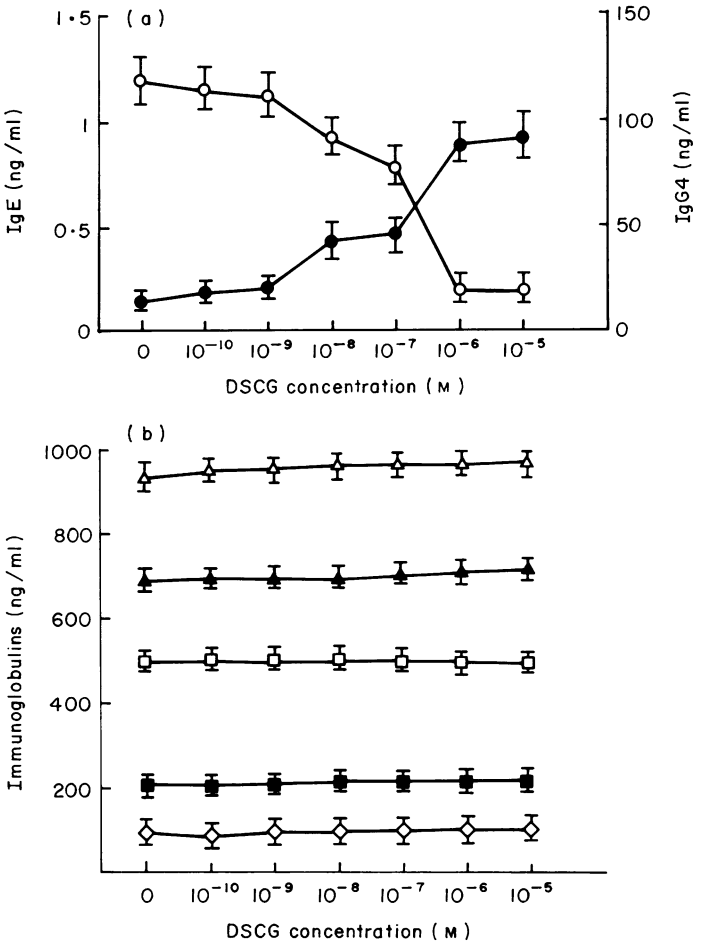

Fig. 1. Effect of DSCG on immunoglobulin production by MNC from non-atopic donors. MNC were stimulated with IL-4 (200 U/ml) in the presence or absence of various concentrations of DSCG. On day 14 of culture, immunoglobulin production was measured. Values are the mean ± 1 s.d. of results from triplicate cultures of: (a) IgE (○) and IgG4 (●); and (b) IgM (△), IgA (▲), IgG1 (□), IgG2 (■), and IgG3 (◇).

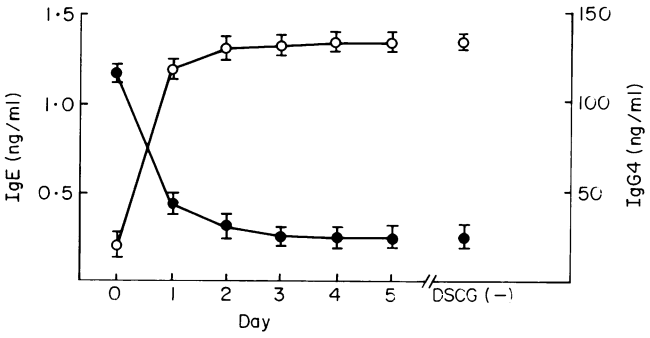

Fig. 2. Kinetic analysis of DSCG addition. MNC from non-atopic donors were stimulated with IL-4 (200 U/ml), and DSCG (10^{-5} M) was added to the cultures daily on days 0–5. As control, IL-4-stimulated MNC were cultured with medium alone, i.e. DSCG(−). On day 14 of culture, immunoglobulin production was measured. Values are the mean ± 1 s.d. of results from triplicate cultures of IgE (○) and IgG4 (●).

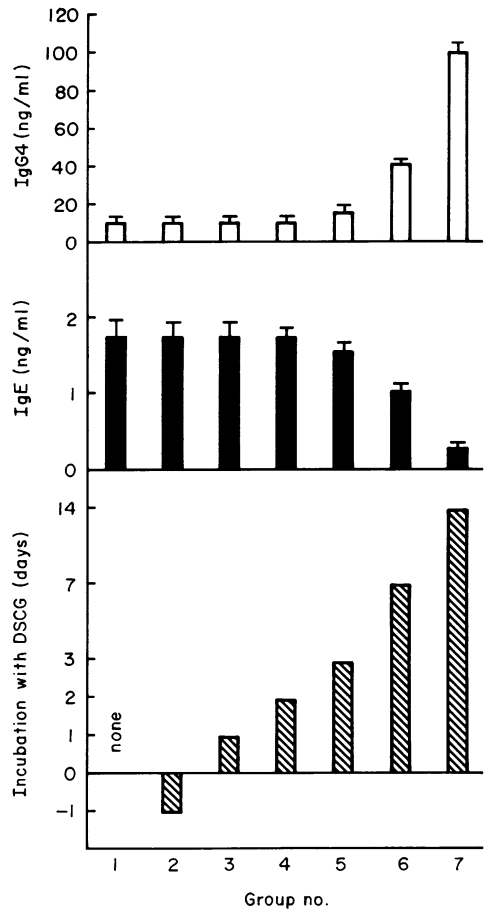

Fig. 3. Effect of incubation with DSCG. MNC from non-atopic donors were divided into following experimental groups: experimental group 1: MNC were cultured with IL-4 for 14 days in the absence of DSCG; experimental group 2: MNC were pre-incubated with DSCG for 1 day, washed and then cultured with IL-4 for 14 days; experimental groups 3–6: MNC were stimulated with IL-4 and incubated with DSCG for the indicated numbers of days, washed and then cultured with IL-4 in the absence of DSCG; experimental group 7: MNC were stimulated with IL-4 and DSCG for 14 days. On day 14 of culture, immunoglobulin production was measured. DSCG was used at 10^{-5} M and IL-4 at 200 U/ml. Values are the mean ± 1 s.d. from triplicate cultures of IgE (■) and IgG4 (□).

Comparison of the effect of DSCG and IFN-γ

IFN-γ has been shown to inhibit IL-4-induced IgE production (Pène *et al.*, 1988; Maggi *et al.*, 1988). The effect of DSCG was then compared with that of IFN-γ in IL-4-stimulated cultures. As shown in Fig. 4, IFN-γ inhibited IgE production without affecting IgG4 production. In contrast, the addition of anti-IFN-γ antibody enhanced IgE production without affecting IgG4 production. Inhibition of IgE production by IFN-γ was blocked by the addition of anti-IFN-γ antibody while isotype control mouse IgG3 failed to do so (data not shown). However, inhibition of IgE production by DSCG was not blocked by the anti-IFN-γ antibody. Moreover, inhibition of IgE production by DSCG and IFN-γ was additive. These results indicate that the mechanism of IgE production inhibition by DSCG is different from that by IFN-γ. On the other hand, neither IFN-γ nor anti-IFN-γ affected IgG4 production. It has also been reported that IFN-α and prostaglandin E_2 (PGE_2) inhibited

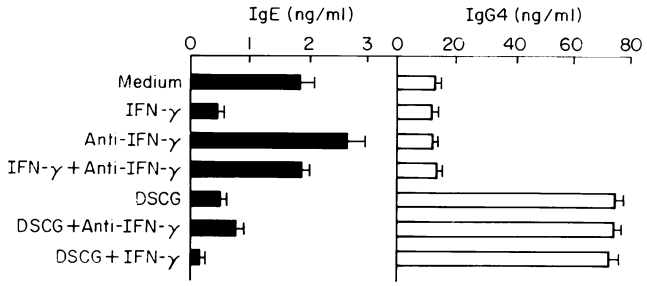

Fig. 4. Comparison of DSCG and IFN-γ. IL-4-stimulated MNC from non-atopic donors were cultured with the indicated factors. On day 14 of culture, IgE and IgG4 production was measured. IFN-γ was used at 100 U/ml, anti-IFN-γ at 10 μg/ml and DSCG at 10^{-5} M. Values are the mean ± 1 s.d. from triplicate cultures of IgE (■) and IgG4 (□).

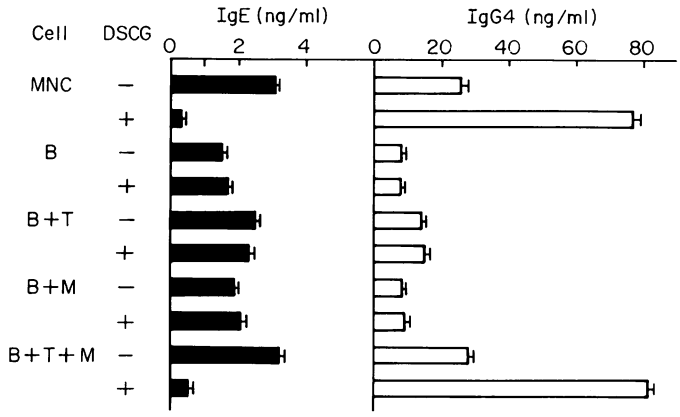

Fig. 5. Effect of DSCG on spontaneous IgE and IgG4 production by cells from atopic patients. MNC (3×10^5/well), B cells (B) ($1{\cdot}5 \times 10^5$/well), B and T cells (T) ($1{\cdot}5 \times 10^5$/well), B cells and monocytes (M) (3×10^4/well), or B cells and T cells and monocytes from atopic patients were cultured as indicated. They were cultured with or without DSCG (10^{-5} M) for 14 days, and IgE and IgG4 production was measured. No IgE or IgG4 production by T cells and/or monocytes alone was detected. Values are the mean ± 1 s.d. from triplicate cultures of IgE (■) and IgG4 (□).

IL-4-induced IgE production (Pène *et al.*, 1988). However, neither IFN-α nor PGE_2 enhanced IgG4 production (data not shown). Therefore, the DSCG effect is unique.

Effect of DSCG on IgE and IgG4 production by atopic B cells

The effect of DSCG on atopic B cells was also studied. As shown in Fig. 5, DSCG inhibited spontaneous IgE production and enhanced IgG4 production by MNC from atopic patients. IgM, IgA, IgG1, IgG2 and IgG3 production was not affected by DSCG (data not shown). In contrast, DSCG did not affect IgE or IgG4 production from purified B cells or from B cells with either T cells or monocytes. However, when B cells were cultured with T cells and monocytes, DSCG inhibited IgE production and enhanced IgG4 production (Fig. 5). As in IL-4-stimulated MNC from normal donors, this effect was observed only when DSCG was added at the initiation of the culturing and present throughout the entire culture period (data not shown).

DISCUSSION

We have demonstrated that DSCG inhibited IgE production and enhanced IgG4 production in IL-4-stimulated MNC from normal donors when added to the culture at the initiation and present throughout the entire culture period. DSCG is well known as anti-allergic medication, and is used for bronchial asthma, allergic rhinitis and allergic conjunctivitis. In addition to that, we have previously reported that topical use of DSCG solution was very effective in the treatment of atopic dermatitis (Kimata & Igarashi, 1990a). Moreover, DSCG inhibited allergen-induced weal and erythema responses *in vivo* (Kimata & Igarashi, 1990b). In patients with asthma, DSCG treatment caused a decrease in house-dust-mite-specific IgE levels (Diaz *et al.*, 1984). DSCG selectively inhibits antibody-dependent granulocyte-mediated cytotoxicity (Rand *et al.*, 1988). These results indicate that in addition to anti-allergic effects of DSCG on basophils and mast cells, DSCG can act on various targets. Interestingly, IgG4 production was enhanced while the production of IgM, IgA, IgG1, IgG2 and IgG3 was not affected. It has been reported that IgG4 acts as a blocking antibody in IgE-mediated allergies and parasite infections (Aalberse *et al.*, 1983). For example, in grass-pollen-allergic patients undergoing specific immunotherapy, IgG4 antibody levels rose during the immunotherapy (Djurup & Osterballe, 1984). In honey-bee-sting-allergic patients and in bee keepers, honey bee venom-specific IgE levels fell while honey bee venom-specific IgG4 levels rose during immunotherapy (Cheung *et al.*, 1983). In patients with *Trichinella spiralis* infection, total IgE levels fell while specific IgG4 levels rose during the course of infection (Ljungström *et al.*, 1988). These *in vivo* results indicate that during the course of various IgE-mediated diseases, there was an inverse relationship between IgE and IgG4 levels. It is possible that one of the therapeutic mechanisms of DSCG *in vivo* may be due to enhancement of IgG4 production which in turn acts as blocking antibody. This possibility is currently under investigation.

IL-4 has been shown to induce IgE and IgG4 production in human spleen and peripheral blood cells (Lundgren *et al.*, 1989; Ishizaka *et al.*, 1990). In contrast to those reports, IL-4 did not induce IgG4 production in tonsil cells (Kimata *et al.*, 1991). It is also of note that in our tonsil cell cultures, IgE production by IL-4 is low (1–2 ng/ml) compared with other reports (Pène *et al.*, 1989). These differences may be due to the cell source, since tonsil cells were the least effective in producing IgE in response to IL-4 (Pène *et al.*, 1989). Alternatively, that may be due to racial difference, since in Japanese donors maximal induction of IgE production by IL-4 is 1·0–1·2 ng/ml even in peripheral blood lymphocytes (Ishizaka *et al.*, 1990).

IFN-γ has been shown to inhibit IL-4-induced IgE production (Pène *et al.*, 1988; Maggi *et al.*, 1988). Although IL-4-induced IgE production was also inhibited by IFN-γ, the mechanism of IgE production inhibition by DSCG is different from that by IFN-γ, for the following reasons. Firstly, inhibition by DSCG was not blocked by the anti-IFN-γ antibody which completely blocked the inhibition of IgE production by IFN-γ. Moreover, inhibitory effects of DSCG and IFN-γ on IgE production were additive. Secondly, DSCG enhanced IgG4 production while IFN-γ did not affect IgG4 production. IL-4-induced IgE production was dependent on the presence of T cell and monocyte (Pène *et al.*, 1988; Maggi *et al.*, 1989). Since MNC

were used, DSCG may stimulate T cells or monocytes to produce some cytokine(s) or factors which in turn inhibit IgE production. IFN-α and PGE_2 have been shown to inhibit IgE production (Pène *et al.*, 1988). In our culture system, IFN-α or PGE_2 inhibited IgE production in IL-4-stimulated tonsil MNC, but did not affect IgG4 production (data not shown). Moreover, DSCG did not stimulate MNC to produce IFN-γ, IFN-α or PGE_2 (unpublished results). Therefore, the factor(s) that affected IgE and IgG4 production remain to be elucidated. Alternatively, it is possible that DSCG affected IgE and IgG4 production through physical contact between cells (see below). This possibility is currently under investigation.

DSCG also inhibited spontaneous IgE production and enhanced IgG4 production by atopic B cells. To produce this effect T cells and monocytes are required. It is possible that DSCG stimulated physical contact between cells which in turn inhibited IgE production and enhanced IgG4 production. Alternatively, DSCG may have stimulated T cells and/or monocytes to produce some factor(s) that modulated IgE and IgG4 production. Preliminary data using double-chamber wells show that if B cells are cultured in one chamber while T cells and monocytes are cultured in another chamber, DSCG does not modulate IgE and IgG4 production. In contrast, when B cells, T cells and monocytes were cultured in the same chamber, DSCG inhibited IgE production and enhanced IgG4 production (manuscript in preparation). Therefore, the effect of DSCG was not mediated by some factor(s) but through physical contact between cells. Whether the same is true for IL-4-stimulated MNC cultures is not known. Studies are currently in progress.

Our results indicate that DSCG is an excellent reagent for the study of human IgE and IgG4 regulation *in vitro*. The results may aid in determining the therapeutic mechanisms of DSCG in IgE-mediated allergic diseases.

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