

Disodium Cromoglycate Inhibits $S\mu \rightarrow S\epsilon$ Deletional Switch Recombination and IgE Synthesis in Human B Cells

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Summary

IgE synthesis requires interleukin 4 (IL-4) and a T-B cell interaction that involves the B cell antigen CD40 and its ligand expressed on activated T cells. IL-4 induces ϵ germline transcription whereas ligation of CD40 results in deletional $S\mu \rightarrow S\epsilon$ switch recombination, expression of mature ϵ transcripts, and IgE synthesis and secretion. We demonstrate that disodium cromoglycate (DSCG), a drug commonly used for the prophylactic treatment of allergic disease, inhibits T cell-driven IgE synthesis by human B cells at concentrations readily achievable in the course of inhaled therapy for asthma. Inhibition of IgE synthesis by DSCG was not the result of drug toxicity because DSCG did not affect the viability of T and B cells or their proliferation to mitogens. DSCG did not interfere with CD40 ligand expression by T cells but clearly targeted the B cells because it inhibited IgE synthesis induced by anti-CD40 and IL-4 in populations of highly purified B cells. DSCG had no effect on the induction of ϵ germline transcripts by IL-4 but strongly inhibited CD40 mediated $S\mu \rightarrow S\epsilon$ deletional switch recombination in IL-4-treated B cells as assayed by nested primer PCR. The effect of DSCG was not specific for CD40-mediated induction of IgE isotype switching because DSCG inhibited IgE synthesis as well as $S\mu \rightarrow S\epsilon$ deletional switch recombination induced by hydrocortisone and IL-4 in B cells. Moreover, the effect of DSCG was not specific for IgE isotype switching because DSCG inhibited the synthesis of IgG₄ by B cells sorted for lack of surface expression of IgG₄ and stimulated with anti-CD40 and IL-4. DSCG caused only minimal inhibition (<15%) of spontaneous IgE synthesis by lymphocytes from patients with the hyper-IgE syndrome and did not affect pokeweed mitogen-induced IgG and IgA synthesis by lymphocytes suggesting that it has little effect on B cells that have already undergone isotype switching. These results indicate that DSCG inhibits switching to IgE in B cells and suggest a novel potential mechanism for the prevention of allergic disease by DSCG.

Disodium cromoglycate (DSCG)¹, the salt of a bis-chromone carboxylic acid, has an established role in the prophylactic treatment of bronchial asthma, allergic rhinitis, and conjunctivitis (1, 2). Despite intensive research, the precise mechanisms by which DSCG exerts its clinical activity are still poorly understood. The primary mode of action of cromolyn sodium was thought to be stabilization of mast cells and subsequent prevention of mediator release after antigen challenge (3-6). More recently, DSCG was shown to inhibit the in vitro activation of human neutrophils, eo-

sinophils, and monocytes (7). Little is known about the effect of DSCG on IgE production except for a single report in which DSCG was found to inhibit spontaneous as well as IL-4-induced IgE production (8).

Induction of IgE synthesis in human B cells requires two signals. The first signal is delivered by the cytokine IL-4 and results in ϵ germline transcription but not in IgE isotype switching (9, 10). A second signal is required for isotype switching and IgE synthesis and secretion. This second signal is normally delivered by T cells (11, 12). We have previously shown that the T cell signal can be replaced by mAb to CD40 (13), Epstein-Barr virus (14), and hydrocortisone (HC) (15). Addition of any of these agents to IL-4-treated B cells results in deletional $S\mu \rightarrow S\epsilon$ switch recombination, expression of mature ϵ transcripts, and IgE synthesis and secretion (16).

Interaction between CD40 and its ligand appears to play an important role in T cell-dependent isotype switching to

¹ Abbreviations used in this paper: AET, 2-aminoethyl isothiuronium bromide; DSCG, disodium cromoglycate; GaMlg, goat anti-mouse IgG; HC, hydrocortisone; PKC, protein kinase C; PTK, protein tyrosine kinase; r, recombinant.

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IgE (10, 17). It has been recently established that activated T cells express a ligand for CD40 (18) and that a soluble form of the CD40 ligand inhibits T cell-driven isotype switching to IgE in IL-4-treated B cells (19). Furthermore, Epstein-Barr virus-transformed B cells transfected with the CD40 ligand replace T cells in synergizing with IL-4 to induce IgE synthesis in B cells (20).

We herein demonstrate that DSCG inhibits IL-4-driven T cell-dependent IgE synthesis by PBMC from the majority (~65%) of normal human subjects as well as IgE synthesis by B cells stimulated with anti-CD40 + IL-4 or with HC + IL-4. DSCG did not interfere with CD40 ligand expression by T cells after stimulation by PMA and ionomycin. It also had no effect on the induction of ϵ germline transcripts by IL-4 but strongly inhibited CD40-mediated deletional switch recombination in IL-4-treated B cells. Furthermore, DSCG inhibited isotype switching in B cells to IgG₄. These results strongly suggest that DSCG acts directly on B cells to inhibit immunoglobulin class switching in B cells.

Materials and Methods

Cell Preparation, Cell Culture, and IgE Assay. Highly purified B cells from nonatopic subjects were prepared as previously described (11). In summary, PBMC from healthy subjects were isolated by density gradient centrifugation, rosetted twice with 2-aminoethylisothiuronium bromide (AET)-treated SRBC, and treated twice with anti-CD3 mAb (OKT3; Ortho Diagnostics, Raritan, NJ) plus rabbit complement (Pel-Freez Biologicals, Rogers, AR). Monocytes were removed by adherence to plastic petri dishes. The B cell-rich populations thus obtained contained <1% CD3⁺ cells, and did not proliferate in response to PHA (10 μ g/ml) but strongly proliferated in response to PMA (25 ng/ml) + ionomycin (0.5 μ M). PBMC were also isolated from two patients with hyper-IgE syndrome whose serum IgE was ~3,000 U/ml and ~10,000 U/ml, respectively.

PBMC and B cells were suspended in RPMI 1640 supplemented with 10% fetal calf serum (Hyclone Sterile Systems, Inc., Logan, UT), 2 mM L-glutamine, 100 U/ml penicillin, and 50 μ g/ml streptomycin (complete medium) in the presence or absence of IL-4 (50 U/ml) and anti-CD40 mAb (5 μ g/ml) or PWM (1 μ g/ml). Control cultures for the evaluation of preformed immunoglobulin were set up in the presence of cycloheximide (100 mg/ml; Sigma Chemical Co., St. Louis, MO). At day 10 of culture, supernatants were assessed for their IgE level by a radioimmunoassay (RIA) with a sensitivity limit of 150 pg/ml (21). IgG₄ levels were determined by a commercially available ELISA assay (The Binding Site, Ltd., Birmingham, UK) with a lower limit of sensitivity of 2.1 ng/ml. IgG, IgA, and IgM levels were determined by ELISA, as previously described (21). Net IgE synthesis was calculated by subtracting the values of preformed IgE.

Reagents. DSCG, obtained as a gift from Fisons Pharmaceuticals (Loughborough, UK), is a disodium salt of 1,3-bis(-2 carboxychromon-5-yloxy) 2-hydroxypropane. DSCG was dissolved in water at 10⁻² M and filtered through a filter (Millipore Corp., Bedford, MA). DSCG was further diluted in complete medium. Human recombinant (r)IL-4 (specific activity 2 \times 10⁷ U/mg) was obtained from Amgen Biologicals (Thousand Oaks, CA) and the anti-CD40 mAb 626.1 has previously been described (22). PHA,

PMA, Con A, AET, and ionomycin were purchased from Sigma Chemical Co. [³H]Thymidine and Econofluor were from New England (Boston, MA). sCD40 and sCD44 were products of fusion of cDNA segments encoding the extracellular domain of CD40 (23) and CD44 (24) to genomic DNA segments encoding human IgG1 (18) and were purified on a protein A column.

Assays for B Cell Proliferation. For proliferation assays, B cells were cultured in 96-well flat-bottomed plates (Nunc, Roskilde, Denmark) at 10⁵ cells/well in 200 μ l complete medium for 72 h in the presence or absence of the indicated stimuli. The cultures were then pulsed with [³H]thymidine at 1 μ Ci/well for an additional 16 h, harvested, and [³H]thymidine incorporated into cellular DNA was counted.

Cell Surface Expression of the CD40 Ligand. T cells, isolated from PBMC by rosetting once with AET-treated SRBC, were left untreated or were stimulated with 20 ng/ml PMA, and 0.5 μ M ionomycin. The cells were then suspended in staining buffer and incubated successively for 30 min on ice with 20 μ g/ml sCD40 or sCD44 as a control, 10 μ g/ml biotinylated Protein G (Calbiochem-Novabiochem Corp., La Jolla, CA), and phycoerythrin-labeled Streptavidin (R & D Systems, Inc., Minneapolis, MN) as previously described (25). Fluorescence was examined using a FACScan[®] (Becton Dickinson & Co., Mountain View, CA).

Cell Sorting. To obtain highly purified B cells, B cell-rich populations of cells were labeled with FITC-conjugated anti-CD19 mAb, and sorted on a FACStar⁺ flow cytometry (Becton Dickinson & Co.). Setting of the markers and analysis of the sorted cells were performed as previously described (13).

Enriched populations of B cells were depleted of surface IgG₄-bearing B cells by magnetic cell sorting using mouse anti-human IgG₄ mAb (The Binding Site, Ltd.) and magnetic beads coated with goat anti-mouse IgG (GaMIg). Depletion of surface IgG₄⁺ cells was monitored by FACS[®] analysis using mouse anti-human IgG₄ mAb and fluorescein-conjugated GaMIg.

Northern Blot Analysis. Total cellular RNA was prepared by cell lysis with guanidium isothiocyanate followed by centrifugation of the lysate through a cesium chloride step gradient. Northern blot analysis was carried out with 10 μ g of total cellular RNA using ³²P-labeled gel purified DNA probes. A 0.88-kb Hinfl fragment that spans the first two exons of C ϵ detects both germline and mature C ϵ transcripts. The human cDNA β -actin probe was kindly provided by Dr. C. Terhorst (Beth Israel Hospital, Boston, MA). Prehybridization, hybridization, washing, and autoradiography conditions were as previously described (13). Densitometric analysis was performed with a densitometer (Ultrascan XL; LKB Instruments, Bromma, Sweden).

PCR Primers/Amplification, Cloning, and Sequencing of S μ /S ϵ "Switch Fragments." High molecular weight DNA was prepared from 5–10 \times 10⁶ cultured B cells using the A.S.A.P. genomic DNA Isolation Kit (Boehringer Mannheim Biologicals, Indianapolis, IN). Nested primer PCR analysis for S μ /S ϵ switch fragments was performed on high molecular weight DNA isolated from cultured B cells using the following four primers as previously described (26): S6:5'-CTGCAGACTCAGAAGGGAGGGGATGCTCCG-3'; S4:3'-ACTGATCCAAGACAGGAGTGTGGCGGATGT-5'; S7:5'-GAGGGTGGTAATGATTTGGTAATGCTTTGGA-3'; S9:3'-GAACCCTGAGGTCCGGTCCCCGCTTCCCCG-5'.

The first round of PCR was performed using primers S6/S4. The second round of PCR was performed on a 5- μ l aliquot of the first round PCR mixture with primers S7/S9. PCR amplification and purification of the amplified fragments were carried out as previously described (26).

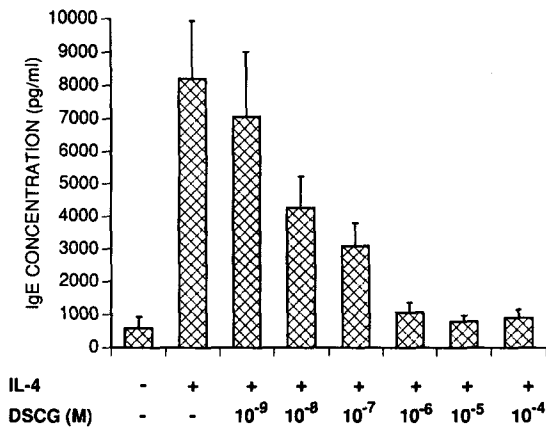


Figure 1. Effect of DSCG on IgE production by PBMC in the presence of IL-4 (50 U/ml). PBMC at a concentration of 1.5×10^6 cells/ml were cultured for 10 d with different concentrations of DSCG. Supernatants were harvested after 10 d and IgE levels were measured by RIA. Values represent mean \pm SE net synthesis IgE (pg/ml) of five experiments.

Results

DSCG Inhibits IL-4-induced IgE Synthesis by PBMC. We first examined the effect of 10^{-6} M DSCG on IgE synthesis induced by IL-4 in PBMC. We chose this concentration of DSCG because it is in the range of the peak DSCG level measured in the serum of patients after inhalation of the drug (7). $1 \mu\text{M}$ DSCG caused virtually complete inhibition (>85% inhibition) of IgE synthesis by PBMC in 11 of 17 subjects studied. In the remaining six subjects inhibition was <50%. Because of this bimodal distribution we focused the remainder of our studies on subjects whose PBMC were susceptible to inhibition by DSCG. Fig. 1 shows that DSCG inhibits IgE synthesis by PBMC stimulated with IL-4 in a dose-dependent manner with 50% inhibition of IgE occurring at a concentration of DSCG of 10^{-8} M.

Induction of IgE synthesis by IL-4 in PBMC is T cell-dependent and involves interaction between the B cell antigen CD40 and the CD40 ligand expressed on activated T cells. DSCG had no effect on either T cell viability as assessed by trypan blue dye exclusion or T cell proliferation to anti-CD3 mAb, PHA, the superantigen TSST-1, and tetanus toxoid

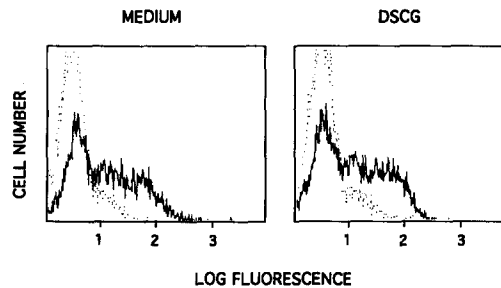


Figure 2. Cell surface expression of the CD40 ligand. T cells were left untreated (dotted line) or stimulated with PMA + ionomycin (solid line). CD40 ligand expression with or without DSCG was assessed at 6 h. There was no detectable binding of sCD44 with or without stimulation (data not shown).

(data not shown). More importantly, DSCG did not interfere with CD40 ligand expression by T cells after stimulation by PMA and ionomycin (Fig. 2). These results suggested that the inhibitory effect of DSCG on IgE synthesis may be exerted at the level of the B cells.

DSCG Inhibits IL-4/CD40-induced IgE Synthesis in B Cells. To determine whether DSCG can exert its effect independent of T cells, we assessed the effect of DSCG on IgE synthesis by a purified B cell population depleted of T cells (<1% CD3⁺ T cells) and stimulated with anti-CD40 mAb and IL-4. Fig. 3 A shows that anti-CD40 mAb 626.1 plus IL-4-induced vigorous IgE synthesis in these B cells. Addition of DSCG resulted in a dose-dependent inhibition of IgE synthesis by B cells. There was ~50% inhibition of IgE synthesis at a concentration of DSCG of 10^{-8} M and >85% inhibition at a concentration of 10^{-4} DSCG.

To unequivocally establish a direct effect of DSCG on B cells, we prepared highly purified B cells by cell sorting for CD19 positive cells. Upon reanalysis, these populations consisted of >98% CD19⁺ cells. Fig. 3 B shows that DSCG inhibited IgE synthesis triggered by anti-CD40 + IL-4 in these B cells. These results indicate that B cells are targets for inhibition by DSCG.

There was no evidence of DSCG toxicity to purified B cells. Fig. 4 shows that these B cells failed to proliferate to PHA but proliferated to PMA + ionomycin and to anti-CD40 +

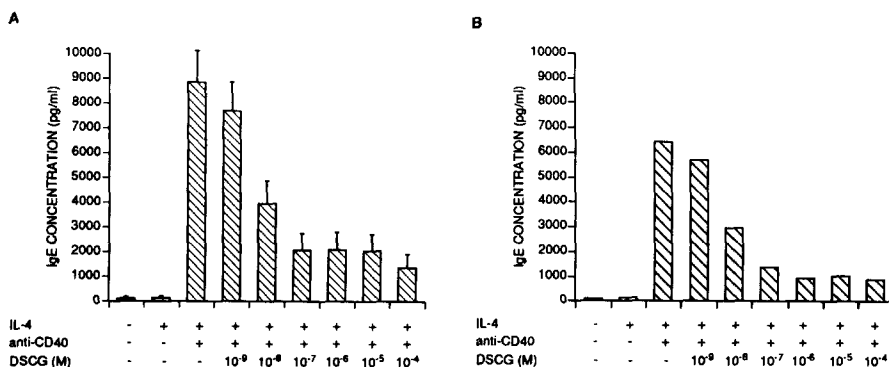


Figure 3. (A) Effect of DSCG on IgE production by T cell-depleted B cell-enriched populations of cells in the presence of IL-4 (50 U/ml) and anti-CD40 mAb (5 $\mu\text{g}/\text{ml}$). Cells were cultured at a concentration of 10^6 cells/ml for 10 d with different concentrations of DSCG. Supernatants were harvested after 10 d and IgE levels were measured by RIA. Results represent mean \pm SE net synthesis IgE (pg/ml) of five experiments. (B) Effect of DSCG on IgE production by highly purified B cells isolated by sorting for CD19 expression. B cells were cultured as described in A. Similar results were observed in a second experiment.

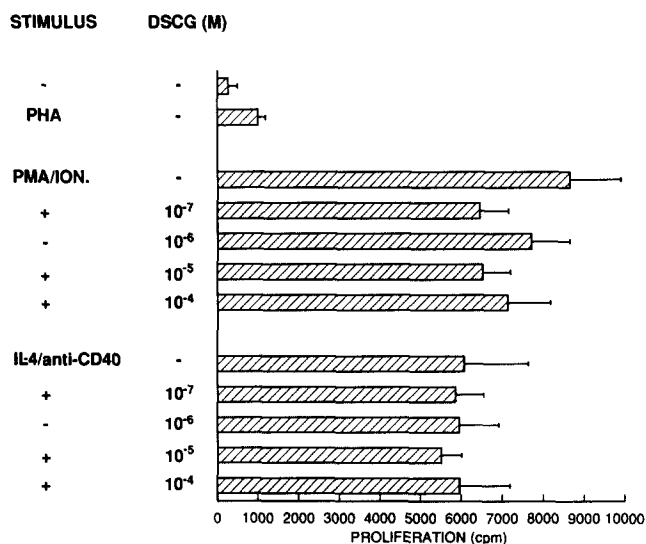


Figure 4. B cells were induced to proliferate by either PMA (20 ng/ml) and ionomycin (0.5 μ M) or IL-4 (50 U/ml) and anti-CD40 mAb (5 μ g/ml). The B cells were cultured for 72 h at 2×10^5 cells/well in 200 μ l in the presence and absence of DSCG. During the last 16 h of culture [3 H]TdR was added to the culture. Results are mean cpm \pm SE of three experiments.

IL-4. Addition of DSCG at concentrations up to 10^{-4} M did not inhibit B cell proliferation. Furthermore, addition of DSCG to B cells stimulated with anti-CD40 + IL-4 to mimic the conditions used for IgE synthesis did not decrease cell viability at the end of the 10-d culture period as assessed by trypan blue dye exclusion (data not shown).

DSCG Does Not Inhibit IL-4 Induction of ϵ Germline Transcripts in B Cells. The effect of DSCG on IgE synthesis could be exerted at the level of induction of ϵ germline transcription by IL-4 or at the level of CD40-mediated switch recombination or at both levels. Fig. 5 A shows that DSCG did not affect the accumulation of ϵ germline transcripts in B cells treated with IL-4. In three experiments, the mean ratio of ϵ germline transcript to actin of B cells stimulated with IL-4 in the presence of DSCG was 1.08 ± 0.08 -fold that of B cells stimulated with IL-4 in the absence of DSCG as determined by densitometry. DSCG by itself did not induce detectable ϵ germline transcripts (data not shown).

We have previously shown that engagement of CD40 by anti-CD40 mAb upregulates ϵ germline transcription induced by IL-4 (26). Fig. 5 B shows that DSCG did not affect the upregulation of IL-4 ϵ germline transcription by anti-CD40. In three experiments, the mean ratio of ϵ germline transcript to actin of B cells stimulated with IL-4 in the presence of DSCG was 1.04 ± 0.09 -fold that of B cells stimulated with IL-4 and CD40 in the absence of DSCG as determined by densitometry. These results strongly suggest that inhibition of IgE synthesis by DSCG is not due to interference with rIL-4 induction of ϵ germline transcripts.

DSCG Inhibits CD40-mediated Induction of Switch Recombination in B Cells. We have previously shown that addition of anti-CD40 mAb to B cells treated with IL-4 results in deletional switch recombination as demonstrated by nested

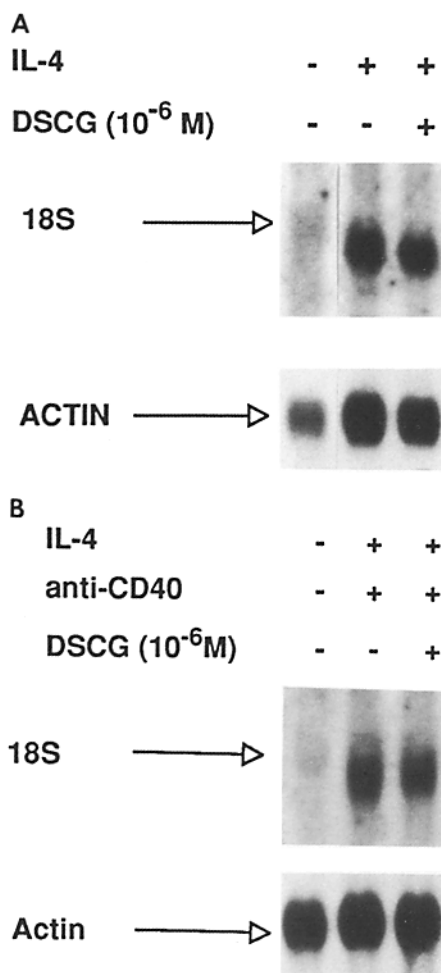


Figure 5. (A) Effect of DSCG on the induction of germline C ϵ transcripts in B cells stimulated with IL-4. Total RNA (10 μ g) was prepared from normal peripheral blood B cells incubated with medium or IL-4 (50 U/ml) or IL-4 (50 U/ml) and DSCG (10^{-6} M) for 5 d then electrophoresed on a 1% formaldehyde-agarose gel, transferred to a nitrocellulose membrane, and hybridized to a 32 P-labeled 0.88-kb HinfI fragment. Densitometric analysis was performed on germline C ϵ transcripts mRNA bands vs. actin transcript mRNA. The autoradiograph was exposed for 5 d. Similar results were obtained in two additional experiments. (B) Effect of DSCG on induction of germline C ϵ transcripts in B cells stimulated with IL-4 and anti-CD40. Total RNA (10 μ g) was prepared from normal peripheral B cells incubated with medium or IL-4 (50 U/ml) plus anti-CD40 mAb (5 μ g/ml) or IL-4 (50 U/ml) plus anti-CD40 mAb (5 μ g/ml) and DSCG (10^{-6} M) for 5 d then proceeded as described for Fig. 6 except that the autoradiograph was exposed for only 3 d. Similar results were obtained in two additional experiments.

primer PCR amplification of recombined S μ /S ϵ regions. To determine whether DSCG interferes with switch recombination, we performed nested primer PCR amplification of recombined S μ /S ϵ regions on DNA isolated from B cells stimulated with IL-4 and anti-CD40 in the presence or absence of DSCG. Fig. 6 shows that 10^{-6} M DSCG strongly inhibited the generation of S μ /S ϵ switch fragments. The presence of a single band in each of the two lanes loaded with the highest amount of DNA from B cells cultured with anti-CD40 + IL-4 and DSCG compared with the multiple bands

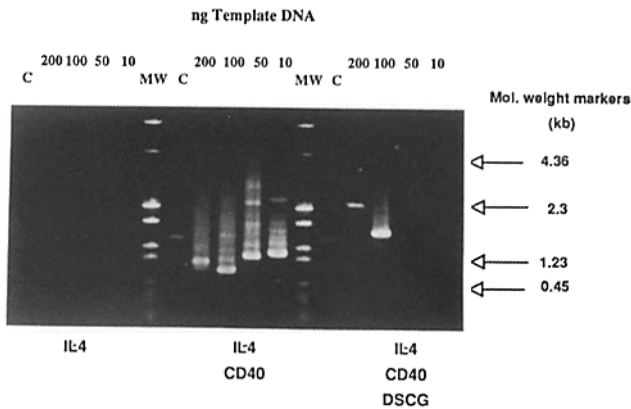


Figure 6. Effect of 10^{-6} M DSCG on the generation of $S\mu \rightarrow S\epsilon$ switch fragments in B cells stimulated with anti-CD40 + IL-4. Serially diluted aliquots of total cellular DNA from B cells stimulated with IL-4, IL-4 plus anti-CD40 mAb, or IL-4 plus anti-CD40 mAb and DSCG (10^{-6} M) were amplified by nested PCR. The serially diluted template DNA sample amounts used in the first round of PCR are noted above the gel. The second round or nested round of PCR used 10% of the original PCR reaction mixture as DNA template. Final PCR products were subjected to agarose gel electrophoresis. PCR amplifiable DNA was present in all three samples, as evident by the control (C) band for which primers specific for the human IL-1 β promoter (-1323/+72) yield a 1.4-kb fragment by PCR. MW, molecular weight markers.

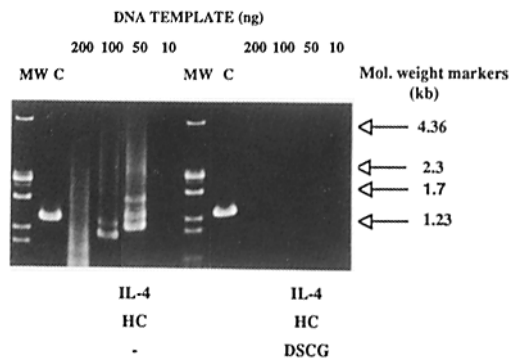


Figure 8. Effect of DSCG on the generation of $S\mu \rightarrow S\epsilon$ switch fragments in B cells stimulated with HC+IL-4. Serially diluted aliquots of total cellular DNA from B cells stimulated with IL-4, IL-4 plus HC, or IL-4 plus HC and DSCG (10^{-6} M) were amplified by nested PCR as described for Fig. 6. PCR amplifiable DNA was present in all three samples, as evident by the control (C) band for which primers specific for the human IL-1 β promoter (-1323/+72) yield a 1.4-kb fragment by PCR. MW, molecular weight markers.

seen in lanes loaded with equivalent amounts of DNA from B cells cultured with anti-CD40 + IL-4 is not unexpected given the fact that in the experiment shown the inhibition of IgE synthesis by DSCG was not complete (2,274 pg/ml in the presence of DSCG vs. 16,348 pg/ml in control cultures; inhibition 86%). These results strongly suggest that DSCG inhibits CD40-mediated deletional switch recombination.

DSCG Inhibits HC-mediated Induction of IgE Synthesis and Switch Recombination in IL-4-treated B Cells. We have previously shown that HC + IL-4 induces IgE synthesis in B cells (15). This is accompanied by deletional switch recombination (27). To determine whether the effect of DSCG on

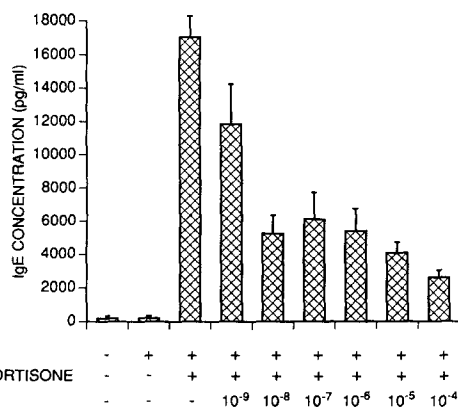


Figure 7. Effect of DSCG on IgE production induced in B cells by hydrocortisone (10^{-6} M) and IL-4 (50 U/ml). Values represent mean \pm SE net synthesis IgE (pg/ml) of three experiments.

IgE isotype switching is restricted to CD40, we examined the effect of DSCG on IgE isotype switching induced by HC + IL-4. Fig. 7 shows that DSCG inhibited IgE synthesis induced by HC + IL-4 in B cells. The dose-response curve of this inhibition was similar to that seen with anti-CD40 + IL-4. Fig. 8 shows that DSCG completely inhibited the generation of $S\mu/S\epsilon$ switch fragments in B cells treated with HC + IL-4. These results suggest that the inhibitory effect of DSCG on IgE isotype switching is not specific to CD40 but targets switch recombination.

DSCG Inhibits Isotype Switching to IgG₄ in B Cells Stimulated with Anti-CD40 and IL-4. Immunoglobulin class switching is thought to involve events that are common to all isotypes as well as events that are isotype specific (28). It was therefore important to determine whether the inhibitory effect of DSCG on isotype switching was restricted to IgE or also extended to other isotypes. Because IL-4 also directs switching to IgG₄, we examined the effect of DSCG on IgG₄ synthesis in B cells stimulated with IL-4 and anti-CD40. To ensure that we were measuring isotype switching, and not merely amplification of immunoglobulin secretion by B cells that had already undergone isotype switching, we used B cells that were magnetically sorted for lack of IgG₄ surface expression. Fig. 9 depicts the FACS[®] analysis of B cells before and after sorting. There was no detectable surface expression of IgG₄ in the negatively sorted B cells. To ensure that the effect of DSCG was not exerted at the level of IL-4 induction of γ_4 germline transcription, we preincubated the B cells for 3 d with IL-4 to allow for optimal expression of germline transcripts, then stimulated the cultures with anti-CD40 with or without DSCG. Fig. 10 shows that unstimulated sIgG₄⁻ B cells secreted no detectable IgG₄ or IgE into their supernatants. Anti-CD40 + IL-4 induced the secretion of IgG₄ and IgE whereas none of these two agents by itself caused IgG₄ or IgE synthesis (data not shown). Because of the low number of B cells recovered post

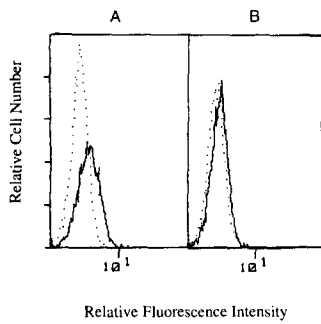


Figure 9. FACS[®] analysis of purified B cells subjected to magnetic cell sorting to deplete IgG₄-bearing cells. A and B show IgG₄ surface expression before and after sorting respectively. The solid line shows B cells stained with mouse anti-human IgG₄ mAb and the dotted line B cells stained with mouse IgG control, both followed by FITC-conjugated GaMlg.

sorting we could test the effect of only two concentrations of DSCG, 10^{-8} and 10^{-6} M. DSCG inhibited the secretion of both IgG₄ and IgE (Fig. 10). In three experiments the mean \pm SD, inhibition of IgG₄ synthesis was $71 \pm 9\%$ with 10^{-6} M DSCG and $49 \pm 15\%$ with 10^{-8} M DSCG. These results suggest that inhibition of isotype switching by DSCG is not restricted to IgE.

Effect of DSCG on Spontaneous IgE Synthesis by B Cells from Patients with the Hyperimmunoglobulin E Syndrome. The hyper-IgE syndrome is a complex disorder characterized by high levels of IgE, recurrent infections, and chronic dermatitis (29). PBMCs from patients hyper-IgE syndrome synthesize large quantity of IgE (30) and their circulating B cells have undergone deletional switch recombination (31). Table 1 shows that addition of up to 10^{-4} M DSCG to PBMC from patients with the hyper-IgE syndrome resulted in only very modest inhibition (<15%) of spontaneous IgE production. These results suggested that DSCG has a minimal effect on IgE synthesis by B cells that have already undergone isotype switching.

DSCG Has No Effect on PWM-induced Synthesis of Immunoglobulins by PBMC. PWM stimulation of PBMC induces T cell-dependent synthesis of IgG and IgA in B cells that have previously undergone switching (Cooper, M. D., personal communication). This is also evidenced by the observation that T cells from patients with X-linked hyper IgM syndrome that fail to induce isotype switching are capable of helping normal B cells to synthesize IgG and IgA in a PWM-driven system (32, 33). Table 2 shows that DSCG had no significant effect on PWM (1 μ g/ml)-induced immunoglobulin secretion by PBMC. These results strongly suggest that DSCG has no effect on immunoglobulin synthesis by B cells that have undergone isotype switching.

Discussion

In this paper we show that DSCG inhibits IgE synthesis in human B cells and that this inhibition resides at the level of deletional switch recombination. These results suggest a novel potential mechanism for the prevention of allergic disease by DSCG.

Susceptibility of normal subjects to the inhibitory effect of DSCG followed a bimodal distribution. In approximately two thirds of the individuals tested, 10^{-6} M DSCG resulted

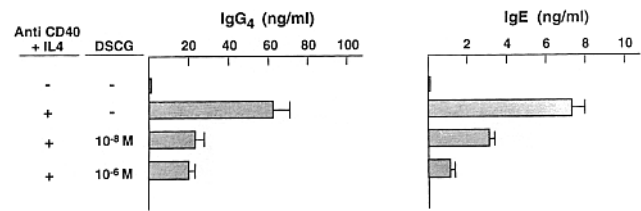


Figure 10. Effect of DSCG on IgG₄ production induced in sIgG₄-B cells by IL-4 (50 U/ml) and anti-CD40 mAb (5 μ g/ml). Cells (10^6 cells/ml) were cultured for 3 d with IL-4. IgG₄ then anti-CD40 was added with or without DSCG for 10 d. IgG₄ and IgE were measured in the supernatants by ELISA and RIA, respectively.

in virtual inhibition of IgE synthesis by IL-4-stimulated PBMC. Inhibition in the remaining one third of the subjects was weak. The reason for this bimodal distribution is at present unclear. It may reflect differences in the ability of cellular receptors to bind DSCG or in the ability of the cells to metabolize the drug.

DSCG inhibited T cell-driven IL-4-dependent IgE synthesis by PBMC in a dose-dependent manner (Fig. 1). Significant inhibition was observed at a concentration as low as 10^{-8} M DSCG (0.5 ng/ml). This concentration is equivalent to that found to inhibit activation of eosinophils, neutrophils, and monocytes (7). More importantly, it is in the range of the peak serum DSCG concentrations (16 ± 5 ng/ml) attained in patients treated with inhaled DSCG (7) and well within the range of DSCG concentration attained locally in the airway after inhalation therapy for asthma (34, 35). These observations together with the decreased antigen-specific IgE in the bronchioalveolar lavage fluid of asthmatics who respond to DSG suggest that DSCG may downregulate IgE synthesis in vivo.

Inhibition of IgE synthesis was not due to toxicity to

Table 1. Effect of DSCG on Spontaneous IgE Synthesis by PBMCs from Patients with Hyper-IgE Syndrome

DSCG	Net IgE synthesis	
	Patient no. 1	Patient no. 2
<i>m</i>	<i>pg/ml</i>	
—	10,380	3,100
10^{-7}	9,392	2,971
10^{-6}	8,841	2,800
10^{-5}	n.a.	2,750
10^{-4}	8,916	2,713

PBMC at a concentration of 1.5×10^6 cells/ml were cultured for 10 d with DSCG at a concentration of 10^{-6} M. Supernatants were harvested after 10 d and IgE levels were measured by RIA. Results for each experiment represent the mean of duplicate cultures. n.a., samples not available for assay.

Table 2. Effect of DSCG on PWM Induction of Immunoglobulin Synthesis by PBMCs

PWM	DSCG	Expt. no. 1			Expt. no. 2		
		IgM	IgG	IgA	IgM	IgG	IgA
	<i>M</i>		<i>ng/ml</i>			<i>ng/ml</i>	
-	-	150	850	90	125	750	75
+	-	3,900	7,100	2,750	3,200	5,000	1,600
+	10 ⁻⁸	4,000	7,200	2,550	2,900	4,800	1,700
+	10 ⁻⁷	3,600	6,900	3,000	3,000	4,300	1,450
+	10 ⁻⁶	4,200	6,900	2,750	3,350	4,700	1,500
+	10 ⁻⁵	3,700	6,700	2,550	2,700	5,100	1,750
+	10 ⁻⁴	3,900	6,900	2,900	3,100	4,900	1,550

PBMC at a concentration of 1.5×10^6 cells/ml were cultured in the presence of PWM (1 μ g/ml) and different concentrations of DSCG. Supernatants were harvested after 10 d and immunoglobulin levels were measured by ELISA. Results for each experiment represent the mean of duplicate cultures.

either T or B cells. Neither the proliferation, nor the viability of T or B cells were affected even at the highest concentrations of DSCG we used (10^{-4} M). The lack of nonspecific toxicity is supported by the finding that DSCG had no effect on PWM-induced immunoglobulin synthesis by PBMC (Table 2).

DSCG did not inhibit the expression of CD40 ligand by T cells stimulated by PMA + ionomycin (Fig. 2). This makes it unlikely that the inhibitory effect of DSCG on T cell-dependent IgE synthesis by PBMC is exerted at the level of the T cell. DSCG clearly targeted the B cells because it inhibited IgE synthesis induced by anti-CD40 and IL-4 in B cell populations (Fig. 3) as well as highly purified B cells that have been positively sorted for the expression of the B cell-specific antigen CD19 (Fig. 4). We cannot, however, rule out an effect of DSCG on T cells, natural killer cells, and monocytes that may contribute to the inhibition by DSCG of IgE synthesis, e.g., via induction of inhibitory cytokines.

DSCG had no effect on the induction of ϵ germline transcription in B cells by IL-4 (Fig. 5). In contrast, DSCG strongly inhibited the CD40-mediated generation of $S\mu \rightarrow S\epsilon$ switch fragments in B cells treated with IL-4 (Fig. 6). These data suggest that DSCG inhibits IgE synthesis in B cells by inhibiting CD40-mediated deletional switch recombination. Given the fact that T cell-driven isotype switching to IgE involves the interaction of CD40 with its ligand on T cells (20), it is likely that DSCG inhibition of T cell-dependent IgE synthesis in PBMC also involves inhibition of CD40-mediated switch recombination in B cells.

Engagement of CD40 results in IL-6 production (36). We have previously shown that IL-6 is required for optimal IgE synthesis because anti-IL-6 antibody strongly inhibited IgE synthesis by PBMC stimulated with IL-4 as well as B cells treated with anti-CD40 + IL-4 (37). It is unlikely that inhibition of IgE synthesis by DSCG was due to inhibition of endogenous IL-6 synthesis because addition of rIL-6 did not

reverse the inhibitory effect of DSCG on IgE synthesis (data not shown).

DSCG did not inhibit all CD40-mediated signals. Neither CD40-mediated B cell proliferation, nor CD40-mediated upregulation of ϵ germline transcript expression induced by IL-4, nor CD40-mediated aggregation of B cells (data not shown) were affected by DSCG. These observations suggest that inhibition of IgE synthesis by DSCG is not specific for the CD40 receptor but targets a discrete step in the CD40 signaling pathway that is required for deletional switch recombination. The observation that DSCG also inhibited IgE synthesis and deletional switch recombination in B cells stimulated with hydrocortisone and IL-4 (Figs. 7 and 8) supports the notion that DSCG does not specifically target CD40-mediated induction of isotype switching.

Common as well as isotype-specific mechanisms are thought to be involved in class switching to different immunoglobulin isotypes (28). The presence of common mechanisms for isotype switching is supported by the finding that patients with X-linked hyperimmunoglobulin M syndrome, who have been recently shown to have deficient expression of the CD40 ligand on their T cells, fail to switch to all isotypes (25). DSCG inhibited isotype switching to IgG₄ induced by CD40 + IL-4 (Fig. 10). This inhibition is unlikely to be due to the effect of DSCG on IL-4-induced γ_4 germline transcription because DSCG inhibited IgG₄ synthesis when added together with anti-CD40 to B cells that were preincubated for 3 d with IL-4 to allow for optimal expression of germline mRNA. Moreover, DSCG did not interfere with two effects of IL-4 we measured directly: ϵ germline transcription and CD23 expression (data not shown). These results suggest that the inhibitory effect of DSCG on isotype switching is not restricted to IgE.

DSCG did not appear to inhibit IgE synthesis in B cells that have already undergone IgE isotype switching. Patients with the hyper-IgE syndrome have in their circulation ter-

minally differentiated B cells that are engaged in IL-4-independent spontaneous IgE synthesis (30) and have circulating B cells that have undergone in vivo switch recombination to IgE (31). IgE production by B cells from these patients was only minimally inhibited (~15%) by DSCG (Table 1). This suggests that DSCG has little effect on IgE synthesis by B cells that have already undergone IgE isotype switching. DSCG had no effect on PWM-induced IgG and IgA synthesis by PBMC (Table 2), a system that does not involve isotype switching (32, 33). This provides further evidence that DSCG has no effect on immunoglobulin synthesis by B cells that have already undergone class switching.

Engagement of CD40 in B cells results in the activation of protein tyrosine kinases (PTK) and of serine/threonine kinases including protein kinase C (PKC) (38). Substrates for PTKs activated in CD40 include the src type kinase *lyn*, phospholipase C- γ 2 and phosphoinositol-3' kinase (39). Inhibition of PTK activity by cross-linking CD45 to CD40 or by genistein results in inhibition of CD40-mediated IgE synthesis and deletional switch recombination (Loh, R., unpublished observation). In contrast, inhibition of PKC activity

by PKC inhibitors has no effect. It will be important to examine the effect of DSCG on PTK activation induced by CD40 engagement. The inhibitory effect of DSCG in rat mast cells is associated with the phosphorylation of a 78-kD protein (3). Phosphorylation of this protein occurs 30–60 s after stimulation of rat peritoneal mast cells and coincides with the termination of the secretory process (40). It remains to be seen whether a protein of similar molecular weight is phosphorylated by DSCG in B cells.

DSCG is a water soluble salt of organic acid with a pKa value between 2 and 3 (41). DSCG is highly ionized at physiological pH values and is consequently unable to penetrate cells (35, 42). It is likely that the action of DSCG involves its association with a membrane receptor. Specific binding of DSCG to rat basophil leukemia cells (RBL-2H3) (3) and binding of DSCG conjugated to polyacrylamide beads to mast cells (43) have been reported. Although these studies support the existence of a membrane receptor for the action of DSCG, isolation and reconstitution of this receptor have not been accomplished. Further work is needed to identify and characterize the putative DSCG receptor.

We thank Dr. Ahern for cell sorting, Dr. D. Vercelli and Dr. T. Chatila for their review of the manuscript, and Dr. A. Edwards for helpful discussions.

This work was supported by National Institutes of Health grant AI-22058.

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Received for publication 27 April 1993 and in revised form 7 March 1994.

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