Disodium Cromoglycate Inhibits Immunoglobulin Production in vitro without Affecting Cell Growth in Human B Cell Lines

H. Hajime Kimata
A. Akira Yoshida
C. Chihiro Ishioka
H. Haruki Mikawa

Department of Pediatrics, Kyoto University Hospital, Kyoto, Japan

Abstract
The effect of disodium cromoglycate (DSCG) on human B cell lines (IM-9, GM-1056, and AF-10) was studied. DSCG inhibited immunoglobulin production by these B cell lines without affecting thymidine uptake or cell number. Thus, in addition to its antiallergic function, DSCG may also act as a B cell-modulating reagent in vitro.

Disodium cromoglycate (DSCG) has been reported to inhibit histamine release from mast cells [1], activation of inflammatory cells including neutrophils, eosinophils, and monocytes [2] and antibody-dependent granulocyte-mediated cytotoxicity [3]. We have previously reported that topical application of DSCG solution was effective in the treatment of atopic dermatitis [4]. Moreover, DSCG inhibited human allergic skin reactions in vivo [5]. These results indicate that DSCG can modulate immune responses both in vitro and in vivo. However, the effect of DSCG on B cell response in vitro has not previously been studied. Thus, the effect of DSCG on human B cell immunoglobulin (Ig) production and thymidine uptake has been studied by using human B cell lines, IM-9 (obtained from the Japanese Cancer Research Resources Bank), GM-1056 (obtained from the NIGMS Human Genetic Mutant Cell Depository, Camden, N.J.) and AF-10 (a kind gift from Dr. Andrew Saxon, UCLA, Los Angeles, Calif.) [6]. These lines produce IgG, IgA, and IgE, respectively [6]. Each cell line was cultured (1 × 10³ / 200 µl/well) in 96-well U-bottom microtiter plates (Coster, Cambridge, Mass.) for 4 days with various concentrations of DSCG in RPMI-1640 medium containing 10% fetal calf serum, 2 mM glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin. The amount of Ig in the culture supernatants was measured by ELISA [7], and cell numbers were counted simultaneously. Cells were also pulsed with 1 µCi [3H]-thymidine 8 h before harvest, and thymidine uptake was measured [7]. As shown in figure 1, DSCG inhibited Ig production from IM-9, GM-1056, and AF-10 at 10⁻⁶ and 10⁻⁵ M (p < 0.01). In contrast, neither thymidine uptake nor cell number was affected by DSCG at any concentration tested (fig. 2). Thus, inhibition of Ig production by DSCG was not due to a decrease in cell growth. It has been reported that in patients on DSCG treatment, IgA concentrations in bronchoalveolar lavage were decreased while there was no decrease in lymphocyte number [8]. This is in agreement with the results of this study: inhibition of IgA production without affecting cell growth. We have previously reported that Ig production from these cell lines was not inhibited by various cytokines including IL-ß, IL-2, IL-4, IL-6, TNF-α,
GM-CSF, IFN-α, IFN-β, and IFN-γ [7]. The mechanisms of this inhibition are now under investigation. DSCG may have acted directly on B cell lines and inhibited Ig production. Alternatively, DSCG may act on B cell lines to produce some inhibitory cytokines, which then inhibit Ig production.

Taken together, these data may indicate that, in addition to its antiallergic function, DSCG may act as a B cell regulatory reagent.

Disodium Cromoglycate Inhibits Immunoglobulin Production

\[ \frac{1}{30} \Gamma + 20 \]

-10

\[ \frac{1}{8} = \frac{1}{8} \]

-30
Fig. 1. Effect of DSCG on Ig production by B cell lines. IM-9 (A), GM-1056 (B), and AF-10 (C) were cultured with various concentrations of DSCG for 4 days, and Ig production was measured. Results are expressed as the mean ± 1 SD of the percent change of control from triplicate cultures. Control Ig production from IM-9, GM-1056, and AF-10 was 9.2 ± 1.0 ng/ml IgG, 28.0 ± 3.1 ng/ml IgA, and 44.2± 1.5 ng/ml IgE, respectively.

Fig. 2. Effect of DSCG on thymidine uptake and cell number by B cell lines. IM-9 (•), GM-1056 (○), and AF-10 (A) were cultured with various concentrations of DSCG for 4 days. Thymidine uptake (A) and cell number (B) were measured. Results are expressed as the mean ± 1 SD of the percent change of control from triplicate cultures. Control thymidine uptake (cpm) by IM-9, GM-1056, and AF-10 was 2,432 ± 246, 3,573 ± 360, and 1,364 ± 179, respectively.

Acknowledgement
This work was supported by a grant from the Ministry of Health and Welfare and a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan.

References