

Effect of disodium cromoglycate on activation of human eosinophils and neutrophils following reversed (anti-IgE) anaphylaxis

R. MOQBEL, G. M. WALSH, A. J. MACDONALD and B. KAY

Department of Allergy and Clinical Immunology, Cardiothoracic Institute, Brompton Hospital, London, SW3, U.K.

(Received 7 August 1985 and in revised form 20 September 1985; accepted for publication 4 October 1985)

Summary.

Immunological release of histamine and lipid mediators is known to occur when basophils, contained in whole blood human leucocytes, are incubated with anti-IgE (reversed anaphylaxis). In the present study we show that IgE-dependent stimulation of basophils was associated with activation of bystander eosinophils and neutrophils, as assessed by enhanced complement (C3b) and IgG (Fc) rosettes, and increased cytotoxicity for complement-coated schistosomula of *Schistosoma mansoni*. These changes in eosinophil and neutrophil function were totally inhibited in a dose-dependent fashion by prior incubation with disodium cromoglycate (DSCG). In all *in vitro* systems examined, complete inhibition of enhancement was observed with concentrations as low as 10^{-7} moles/l. In contrast, DSCG had no effect on histamine release, or the percentage of rosettes or cytotoxicity prior to anti-IgE stimulation. These results suggest that DSCG inhibits activation of inflammatory cells consequent to an IgE-dependent stimulus.

Introduction

Incubation of whole human leucocytes with anti-IgE is associated with a basophil-dependent release of histamine [1] and other mediators, including leukotrienes [2]. We recently showed that stimulation of peripheral blood leucocytes with anti-IgE resulted in enhanced cytotoxicity for schistosomula of *Schistosoma mansoni* by bystander eosinophils and neutrophils contained in these cell suspensions [3]. In a separate series of experiments we also established that activation of eosinophils and neutrophils by the synthetic chemoattractant, N-formyl-methionyl-leucyl-phenylalanine (fMLP), was inhibited by low concentrations of the anti-allergic agent, disodium cromoglycate (DSCG) [4]. This suggested that DSCG may have direct actions on inflammatory cells which are distinct from its ability to stabilise the mast cell.

In the present study we have examined the effects of DSCG on IgE-dependent activation of eosinophils and neutrophils, as shown by changes in C (C3b) and IgG (Fc) rosettes and cytotoxicity. Our findings suggest that products of IgE-dependent allergic responses activate bystander granulocytes and that DSCG inhibits these effects. Thus the drug may modify allergic inflammation by its direct effect on granulocytes.

Materials and methods

Materials were obtained as follows: Iscoves modification of Dulbecco's medium (pH 7.35) with added glutamine and containing 25 mM HEPES and 3.024 g/l sodium bicarbonate and RPMI 1640 with 25 mM HEPES (Flow Laboratories Ltd., Irvine, U.K.); Trizma 7.7, histamine dihydrochloride, pepsin, trypsin and trypsin inhibitor (Sigma Ltd., Poole, U.K.); calcium chloride, magnesium chloride, EDTA and perchloric acid (BDH Chemicals Ltd., Poole, U.K.); analytical grade metrizamide (Nyegaard A/S, Birmingham, U.K.); 6% dextran in 0.9% saline (Lomodex 70, Fisons Pharmaceuticals, Loughborough, U.K.). Rabbit anti-human IgE and normal rabbit immunoglobulin fraction were obtained from Dako (Mercia-Brocades Ltd., Weybridge, U.K.). IgG1 human myeloma protein was a kind gift from Dr D. Stanworth.

Preparation of anti-IgE F(ab')₂

The preparation of anti-human IgE F(ab')₂ has been described previously [5] and was briefly as follows. Rabbit anti-human IgE (anti-IgE) or normal rabbit gammaglobulin (control Ig) were dialysed in 0.1 M sodium acetate and adjusted to pH 4 prior to digestion with pepsin (0.2 mg/10 mg of IgG) for 18 hr. The supernatant was readjusted to pH 7.4 and dialysed against PBS and the F(ab')₂ purified by Sephadex G-100 chromatography. The amount of protein in the pooled F(ab')₂ fraction peak was adjusted to a concentration of 3.25–3.50 mg/ml and its identity and purity determined by immunoelectrophoresis against an antiserum to whole rabbit Ig.

Preparation of Schistosomula

A Puerto Rican strain of *S. mansoni* was maintained in laboratory-bred *Biomphalaria glabrata* and C57/BL mice as described by Smithers & Terry [6]. Schistosomula were mechanically prepared *in vitro* from cercariae according to the method of Ramalho-Pinto *et al.* [7] and were used in the assay system within 6 hr of transformation [8]. Schistosomula were washed (three changes) with Iscoves medium while incubating for 3 hr at 37°C in an atmosphere of 5% CO₂ and 95% air. The number of schistosomula was adjusted to provide a count of 10³ larvae/ml.

Preparation of effector cell suspensions

Blood from normal healthy donors, or from individuals with peripheral eosinophilia of greater than 7% who were not receiving medication, was drawn into tubes containing 0.1 ml of 0.1 M EDTA and 0.25 ml of 6% dextran per ml of blood and left to sediment for 45–60 min at room temperature. Leucocytes were washed twice in Tris+albumin (Tris-A) containing 4% 0.1 M EDTA (Tris-A-EDTA). The cells were finally resuspended in Tris-ACM containing 1 mM CaCl₂ and MgCl₂ and aliquots (approximately 5 × 10⁷ moles/l) of leucocytes were layered on to a discontinuous metrizamide (MTZ) gradient [9]. This technique yielded 90–100% pure eosinophils and neutrophils. Basophils were present only in the interface which contained the mononuclear cells.

Total cell counts and percentage of eosinophils present were determined using Kimura strain [10]. The number of basophils was assessed using both wet counts stained with Alcian blue [11] and fixed smears in which $2\ \mu\text{l}$ of cells from a counted suspension were deposited on a slide, air-dried, fixed and stained with toluidine blue.

Schistosomula killing assay

The details of assay have been described elsewhere [12], but are briefly as follows. The assay was performed in flat-bottomed microtitre plates (Nunc/Gibco, Paisley, U.K.) and the total reaction volume was $0.2\ \text{ml}$. This included $50\ \mu\text{l}$ of schistosomula (fifty larvae) and $50\ \mu\text{l}$ of autologous fresh serum (as a source of complement C) (final dilution of 1:4). To this was added $100\ \mu\text{l}$ containing $50\ \mu\text{l}$ of leucocytes plus $50\ \mu\text{l}$ of either anti-IgE, Ig control or buffer. Schistosomula were also incubated with heat-inactivated serum (56°C for 60 min) in the presence or absence of cells and stimulant, C-alone, cells alone or medium alone. All were adjusted to the same total of $200\ \mu\text{l}$ per well. The plates were incubated for 40 hr (unless otherwise stated) at 37°C in an atmosphere of 5% CO_2 , 95% air and 95% humidity. The effector cell:target ratio was 2000:1, unless otherwise stated.

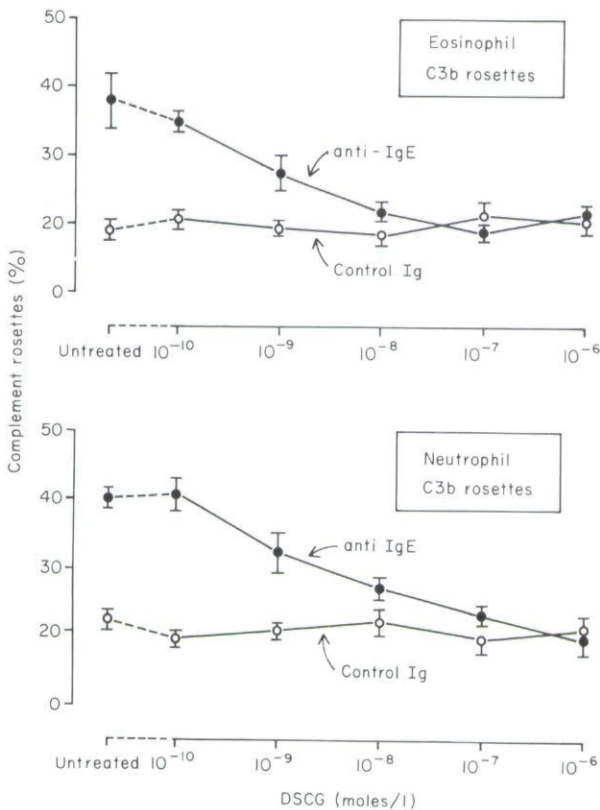


Fig. 1. Percentage complement (C3b) rosettes on eosinophil- and neutrophil-rich cell suspensions containing basophils in the presence or absence of a dose range of DSCG. Cells were incubated with DSCG for 30 min prior to adding anti-IgE or control Ig at a final dilution of 1:500. Granulocytes were purified on MTZ, treated with anti-IgE (or control Ig) in the presence or absence of DSCG. Cells were used at a concentration of $2 \times 10^6/\text{ml}$. The results represent mean $\pm$ s.e.m. of three experiments.

Schistosomular death was measured in a visual assay using the criteria of loss of motility, internal coarse granulation of larvae and the vital exclusion of 0.1% trypan blue dye. These visual criteria have been shown to present unequivocal evidence of larval death when compared to ultrastructural changes [13] and loss of infectivity following injection *in vivo* [14].

Neutrophil and eosinophil rosette assays

After challenge with anti-IgE (or control Ig), whole leucocyte suspensions containing a mixture of eosinophils and neutrophils were used in rosette assays for complement (C3b) and Fc (IgG1) receptors as follows. C3b-coated sensitised sheep erythrocytes (Ea_MC3b) were prepared as previously described [15]. Neutrophil and eosinophil Fc receptor expression was measured using sheep erythrocytes coated with a human IgG1 myeloma protein [16]. The IgG (Fc) rosette assay was performed according to the method described previously [17]. At least 200 neutrophils and eosinophils were counted for both C3b and Fc rosettes.

Stimulation of cells with anti-IgE and control Ig

Leucocytes were incubated with varying dilutions of anti-IgE or control Ig for 30 min at 37°C after which an aliquot was removed from EaC and I-IgG rosette assays. This time has been shown to be optimal for the anti-IgE effect [3]. DSCG: Intal (10 mg/ml DSCG aqueous nebuliser solution; Fisons Ltd., Loughborough, U.K.) was used for inhibition studies. The leucocytes were first incubated for 30 min at 37°C with various molar concentrations of DSCG; a dose of 10^{-7} M has been shown to give maximum inhibition [18]. Incubation with anti-IgE or control Ig and the subsequent rosette and schistosomula killing assays were performed in duplicate as above.

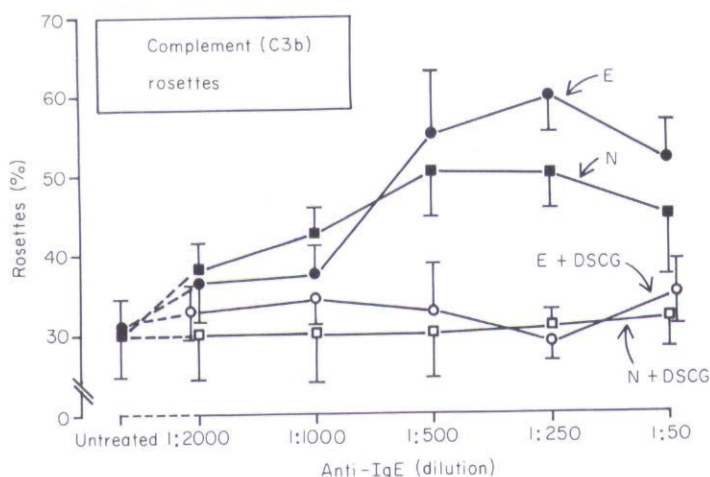


Fig. 2. Percentage eosinophil and neutrophil complement (C3b) rosettes following treatment with a dose range of anti-IgE ($n=3$). Cells were incubated for 30 min in the presence or absence of DSCG at a dose of 10^{-7} moles/l prior to anti-IgE stimulation. Cells treated with the same dose range of control Ig did not show any increase in C3b rosette values beyond that of untreated cells. Closed circles = eosinophils (no DSCG); open circles = eosinophils (+ DSCG); closed squares = neutrophils (no DSCG); open squares = neutrophils (+ DSCG).

Measurement of histamine

Histamine in supernatants and cell pellets of anti-IgE- or control Ig-treated leucocytes was extracted in 5% perchloric acid and measured as amount released (measured in ng) and percentage release using an automated spectrofluorometric (auto-analyser, Technicon) assay [19], in which values were compared with a standard curve of known amounts of histamine dihydrochloride.

Results

The effect of increasing concentration of DSCG on IgE-dependent increases in eosinophil and neutrophil complement rosettes is shown in Fig. 1. An optimal concentration of anti-IgE (1:500) gave approximately 100% increase in eosinophil and neutrophil complement rosettes. Prior incubation with DSCG resulted in a dose-dependent inhibition of enhancement with greater than 90% inhibition being observed with a concentration of 10^{-8} moles/l. DSCG had no effect on the baseline (control Ig) percentage of eosinophil and neutrophil rosettes shown by unstimulated cells.

The effect of an optimal inhibiting dose of DSCG (10^{-7} moles/l) on complement receptor expression following stimulation of leucocytes with varying concentrations of anti-IgE are shown in Fig. 2. Increasing concentrations of anti-IgE were associated with increased neutrophil and eosinophil complement (C3b) rosettes which at all dilutions were inhibited by prior incubation with DSCG.

A similar experiment was undertaken with IgG (Fc) rosettes (Fig. 3). There was a similar increase in neutrophil IgG (Fc) rosettes with maximal enhancement occurring at a 1:250 dilution of anti-IgE. Eosinophil Fc rosettes were also significantly enhanced ($P < 0.05$) at 1:250 dose of anti-IgE, albeit at a considerably lesser extent when compared with the enhancement of neutrophil Fc rosettes. Prior incubation with DSCG inhibited the enhancement of rosettes on both neutrophils and eosinophils.

The effect of increasing concentrations of DSCG on IgE-dependent enhancement

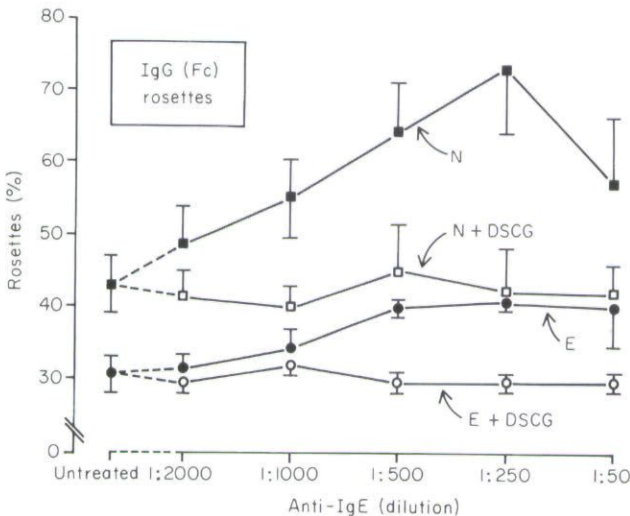


Fig. 3. Percentage eosinophil and neutrophil IgG (Fc) rosettes following treatment with a dose range of anti-IgE ($n = 3$). Closed circles = eosinophils (no DSCG); open circles = eosinophils (+ DSCG); closed squares = neutrophils (no DSCG); open squares = neutrophils (+ DSCG).

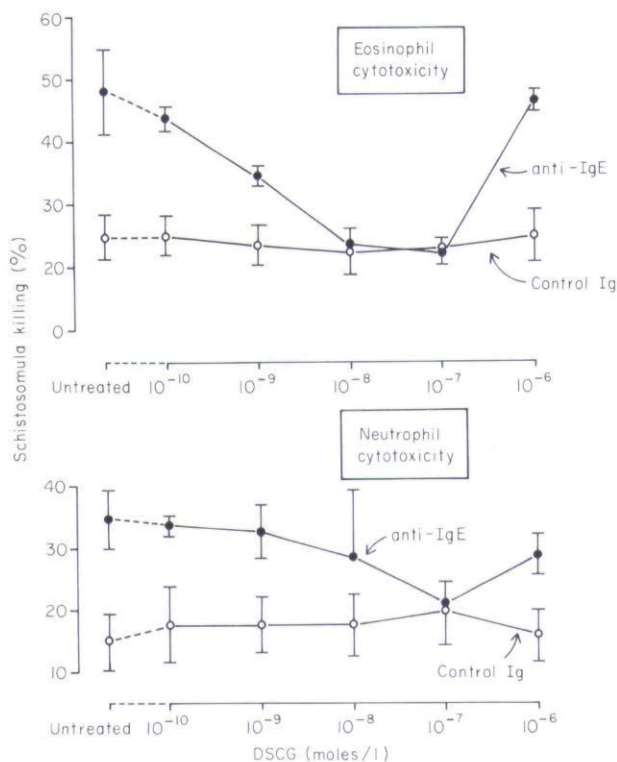


Fig. 4. Percentage killing of complement-coated schistosomula of *S. mansoni* by granulocytes following anti-IgE treatment of eosinophil- and neutrophil-rich cell suspensions containing basophils in the presence or absence of a dose range of DSCG. Cells were incubated with DSCG for 30 min prior to adding anti-IgE or control Ig at a final dilution of 1:500. Granulocytes were purified on MTZ, treated with anti-IgE (or control Ig) in the presence or absence of DSCG and added to C-coated (1:4) schistosomula at a cell:target ratio of 1:4000. The assay was measured at 40 hr. The results represent mean $\pm$ s.e.m. of three experiments.

of eosinophil- and neutrophil-mediated complement-dependent cytotoxicity against schistosomula of *S. mansoni* is shown in Fig. 4. Again there was a dose-dependent inhibition of IgE-dependent enhancement in cytotoxicity with both cell types, which was optimal at 10^{-7} moles/l DSCG. In contrast, there were no significant changes in cytotoxicity observed following control Ig treatment over the whole concentration range of DSCG. Under similar conditions, eosinophil-mediated cytotoxicity was, in general, significantly ($P < 0.01$) higher than that achieved by neutrophils.

DSCG inhibited the enhancing effect of anti-IgE stimulation and reduced the values of both rosettes and cytotoxicity observed following IgE-dependent stimulation to baseline values obtained in control Ig-treated cells. Pretreatment of neutrophil- or eosinophil-rich cell populations with DSCG at the optimal dose of 1×10^{-7} M did not however influence the percentage of histamine released from the basophils found in the cell suspension following anti-IgE stimulation (Table 1). The capacity of normal eosinophils and neutrophils to respond to a dose range of exogenously added histamine dihydrochloride was compared with anti-IgE and control Ig stimulation, and the results are presented in Table 2. Both C3b rosettes and cytotoxicity of both cell types were enhanced by anti-IgE, but only eosinophil-rich preparations were activated

Table 1. Percentage histamine release measured in eosinophil- and neutrophil-rich suspensions containing basophils

Dilutions of anti-IgE	Histamine (% Release)			
	Eosinophil-rich suspension		Neutrophil-rich suspension	
	No DSCG	+DSCG	No DSCG	+DSCG
1:50	56 ± 6	54 ± 2	70 ± 3	64 ± 2
1:250	53 ± 5	54 ± 3	67 ± 2	62 ± 2
1:500	52 ± 10	46 ± 2	65 ± 2	60 ± 2
1:1000	51 ± 12	46 ± 3	50 ± 5	57 ± 2
1:2000	34 ± 3	30 ± 1	36 ± 6	45 ± 4

DSCG was used at a single dose of 10^{-7} moles/l prior to anti-IgE or control Ig stimulation. Control Ig-mediated histamine release in the presence or absence of DSCG pretreatment was also measured in all cell suspensions and did not exceed 10% at each concentration. The results represent the mean $\pm$ s.e.m. of three experiments.

Table 2. Percentage change in schistosomula killing by, and C3b rosette on, eosinophils and neutrophils following anti-IgE or control Ig treatment compared with exogenously-added histamine dihydrochloride

	Concentration	Eosinophils		Neutrophils	
		Cytotoxicity	C3b Rosettes	Cytotoxicity	C3b Rosettes
Anti-IgE	1:250	82.0 ± 9.0	91.7 ± 16.8	122.5 ± 5.5	74.5 ± 14.0
	1:500	48.0 ± 8.7	76.3 ± 23.0	115.5 ± 26.0	72.0 ± 18.5
Control Ig	1:500	32.0 ± 3.0	32.5 ± 3.0	20.0 ± 3.0	30.0 ± 5.5
Histamine	10^{-5} M	40.0 ± 13.0	49.0 ± 26.0	11.0 ± 12.0	40.0 ± 18.0
	10^{-6} M	74.0 ± 18.0	50.0 ± 17.0	19.0 ± 18.0	20.0 ± 3.0
	10^{-7} M	120.0 ± 12.0	63.0 ± 20.0	9.0 ± 22.0	25.0 ± 5.0
	10^{-8} M	14.0 ± 13.0	85.0 ± 15.0	2.0 ± 13.0	19.0 ± 5.0

The results represent the mean $\pm$ s.e.m. of three experiments.

(C3b and cytotoxicity) by histamine in a dose-dependent fashion. Neutrophil C3b rosettes and cytotoxicity were not influenced by prior treatment with histamine.

Discussion

The present results indicate that DSCG inhibits activation of human leucocytes, in addition to its established rôle in stabilising mast cell membranes. Histamine release from basophils, however, is unaffected by DSCG [20], and this was confirmed in the present study (Table 1). Nevertheless, DSCG inhibited the concomitant enhancement of receptor expression and cytotoxicity by eosinophils or neutrophils. DSCG, however, did not suppress or ablate cell function as demonstrated by normal baseline

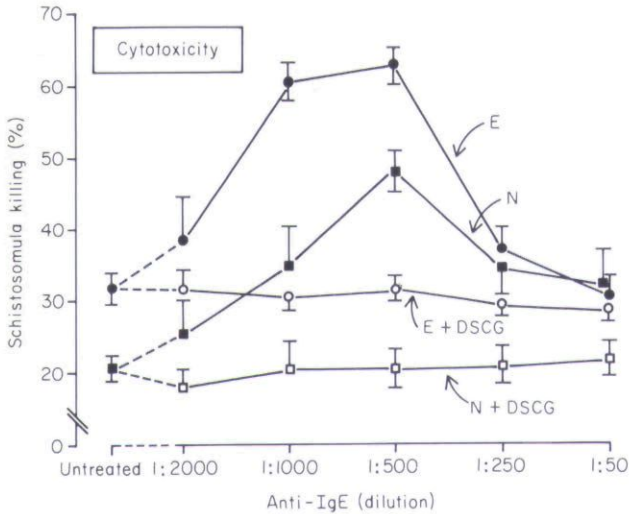


Fig. 5. Percentage killing of complement-coated schistosomula of *S. mansoni* by eosinophils and neutrophils following treatment with anti-IgE ($n=3$). Cells were incubated for 30 min in the presence or absence of DSCG (at a dose of 10^{-7} moles/l) prior to anti-IgE stimulation. Cells treated with the same dose range of control Ig did not show any increase in cytotoxicity beyond that of untreated cells. Closed circles = eosinophils (no DSCG); open circles = eosinophils (+ DSCG); closed squares = neutrophils (no DSCG); open squares = neutrophils (+ DSCG).

values of rosetting and cytotoxicity observed with unstimulated cells following cell pretreatment with the drug.

Our observation that DSCG may inhibit chemotactic factor-induced cell activation [4,18] has been further supported by *in vivo* studies. Treatment of exercise-induced asthma (EIA) patients with DSCG prior to undergoing an exercise task results in the inhibition of both the increase in their airway obstruction (fall in FEV_1) and the observed enhancement in both cytotoxicity [21], as well as C3b receptor expression [22] by leucocytes from these patients, which occurs in the absence of DSCG treatment. Aside from its recognized rôle in stabilizing mast cell membrane, the precise mode of action of DSCG on cell activation is uncertain [23]. Rhodes [24] reported inhibition of lysosomal enzyme release from human neutrophils following challenge with zymosan on aggregated gammaglobulin. Recent studies on rat leukaemic basophil cell line have suggested that DSCG may act by blocking Ca^{2+} flux [25]. This may also involve changes in cyclic adenosine monophosphate (cAMP) levels [18].

In this study we have employed the rosette technique to measure CRE after IgE-dependent stimulation, in preference to a radio-ligand binding assay using a monoclonal antibody (E11) against complement receptor 1 (CR1) [26]. Using the latter technique, we were unable to observe DSCG-mediated inhibition of enhancement of anti-CR1 uptake by activated neutrophils which was demonstrable by parallel rosette experiments [4]. This discrepancy suggests that C3b rosetting may not be dependent exclusively on the number of CR1 molecules on human neutrophils following activation.

The schistosomula cytotoxicity assay was used to measure changes in the functional properties of human leucocytes following IgE-dependent stimulation. The observed enhancement in the capacity of human eosinophils and neutrophils to kill C-

coated schistosomula of *S. mansoni* *in vitro* may be related to the direct effects of basophil-derived mediators released by this reverse-type anaphylaxis on the surface expression of C3b receptors on treated cells, which is reflected in the increased number of specific rosettes. Similar effects were previously reported when chemotactic factors (e.g., histamine and LTB₄) were added exogenously to eosinophils and neutrophils [27,30].

Analysis of the pharmacological contents of the supernatant from anti-IgE-treated leucocytes containing basophils has revealed that, in addition to histamine (already shown here), membrane-derived mediators (leukotrienes, LTB₄ and LTC₄) were also generated [31]. While LTC₄ showed no enhancing activity on both cell types, LTB₄ appeared to enhance neutrophil, and to a lesser extent eosinophil, cytotoxicity; histamine appeared to preferentially enhance eosinophil activation. It is worth noting that pretreatment of human leucocytes with DSCG did not influence the IgE-mediated generation of histamine (Table 1) and LTB₄ and LTC₄ (Moqbel & MacDonald, unpubl. data). Cell activation (i.e., enhanced cytotoxicity, C3b and Fc receptor expression) described here may be attributed to histamine and LTB₄ (for eosinophils) and LTB₄ (for neutrophils). Of course the possible contributions of other inflammatory mediators cannot be excluded.

The enhancement of IgG (Fc) receptors (Fig. 3) may be directly related to the increase in cytotoxicity against IgG-coated schistosomula shown to occur following anti-IgE stimulation of human leucocytes [3]. In addition to IgG receptors, eosinophils have been recently shown to possess low affinity IgE (Fc) receptors [32]. Whether anti-IgE stimulation in this system also causes the enhancement of IgE receptors on eosinophils is unknown and this is presently under investigation.

In conclusion, our results suggest that IgE DSCG down-regulated the chemotactic factor-induced activation of both eosinophils and neutrophils, possibly by inhibiting the enhancement of their receptor expression (C3b and IgG (Fc)), thus influencing their capacity to kill opsonized helminthic targets. This observation, which is important in terms of the specific properties of their anti-allergic agent, may also have *in vivo* implications in modulating immediate-type hypersensitivity reactions associated with the patho-physiology of helminthic infections.

Acknowledgments

This research was supported by The Medical Research Council.

References

- 1 Marone G, Kagey-Sobotka A, Lichtenstein LM. IgE-mediated histamine release from human basophils: differences between antigen E and anti-IgE-induced secretion. *Int Arch Allergy Appl Immunol* 1981; 65: 339-48.
- 2 MacGlashan DW Jr, Schleimer RP, Peters SP, *et al.* Comparative studies of human basophils and mast cells. *Fed Proc* 1983; 42: 2504-9.
- 3 Moqbel R, MacDonald AJ, Kay AB. Enhancement of human eosinophil- and neutrophil-mediated killing of schistosomula of *Schistosoma mansoni* by reversed-type (IgE-mediated) anaphylaxis, *in vitro*. *Clin Exp Immunol* 1985; 59: 577-84.
- 4 Richerson HB, Walsh GM, Walport MJ, Moqbel R, Kay AB. Enhancement of human neutrophil complement receptors: a comparison of the rosette technique with the uptake of radiolabelled anti-CR1 monoclonal antibody. *Clin Exp Immunol* 1985; 62: 442-8.
- 5 Hudson L, Hay FC. In: *Practical Immunology*. London: Blackwell Scientific Publications, 1980: 196-9.
- 6 Smithers SR, Terry RJ. The infection of laboratory host with cercaria of *Schistosoma mansoni* and the recovery of the adult worms. *Parasitology* 1965; 55: 695-700.

- 7 Ramalho-Pinto FJ, Gazzinelli G, Howells RE, Mota-Santos TA, Figueiredo EA, Pellegrino J. *Schistosoma mansoni*: defined system for stepwise transformation of cercaria to schistosomula *in vitro*. *Exp Parasitol* 1974; 36: 360–72.
- 8 MacDonald AJ, Moqbel R, Kay AB. The effect of age on the susceptibility of schistosomula of *Schistosoma mansoni* to damage by human eosinophils and neutrophils, *in vitro*. *J Parasitol* 1985; (in press).
- 9 Vadas MA, David JR, Butterworth AE, Pisani NT, Siongok TA. A new method for the purification of human eosinophils and neutrophils and a comparison of the ability of these cells to damage schistosomula of *Schistosoma mansoni*. *J Immunol* 1979; 122: 1228–36.
- 10 Kimura I, Moritani Y, Tanizaki Y. Basophils in bronchial asthma with reference to reagin-type allergy. *Clin Allergy* 1973; 3: 195–202.
- 11 Gilbert HS, Ornstein L. Basophil counting with a new staining method using alcian blue. *Blood* 1975; 46: 279–86.
- 12 Anwar ARE, Smithers SR, Kay AB. Killing of schistosomula of *Schistosoma mansoni* coated with antibody and/or complement by human leucocytes *in vitro*: requirement for complement in preferential killing by eosinophils. *J Immunol* 1979; 122: 628–37.
- 13 McLaren DJ, Ramalho-Pinto FJ, Smithers SR. Ultrastructural evidence for complement and antibody-dependent damage to schistosomula of *Schistosoma mansoni* by rat eosinophils *in vitro*. *Parasitology* 1978; 77: 313–24.
- 14 Dessein A, Butterworth AE, Vadas MA, David JR. Maturation *in vivo* of *Schistosoma mansoni* schistosomula after culture *in vitro* with granulocytes and antibody. *Infect Immun* 1983; 39: 225–32.
- 15 Carroll M, Durham SR, Walsh GM, Kay AB. Activation of neutrophils and monocytes after allergen- and histamine-induced bronchoconstriction. *J Allergy Clin Immunol* 1985; 75: 290–6.
- 16 Gonzalez-Molina A, Spiegelberg HL. A subpopulation of normal human peripheral B-lymphocytes that bind IgE. *J Clin Invest* 1977; 59: 616–24.
- 17 Kay AB, Walsh GM. Chemotactic factor-induced enhancement of the binding of human immunoglobulin classes and subclasses to neutrophils and eosinophils. *Clin Exp Immunol* 1984; 57: 729–34.
- 18 Kay AB, Nagakura T, Walsh G, Carroll M. Disodium cromoglycate inhibits leucocyte activation *in vitro*. *J Allergy Clin Immunol* 1984; 73: 170 (abstr.).
- 19 Siraganian RP, Brodsky MJ. Automated histamine analysis for *in vitro* allergy testing. *J Allergy Clin Immunol* 1976; 57: 525–40.
- 20 Church MK. The role of basophils in asthma. I. Sodium cromoglycate on histamine release and content. *Clin Allergy* 1982; 12: 223–8.
- 21 Kay AB, Durham SR, Gin W, et al. Inflammatory cells in early, late-phase and chronic asthma. In: *Asthma and Bronchial Hyperreactivity* (Progress in Respiration Research, Vol. 19). Basel: S. Karger, 1985: 211–23.
- 22 Papageorgiou N, Carroll M, Durham SR, Lee TH, Walsh GM, Kay AB. Complement receptor enhancement as evidence of neutrophil activation after exercise-induced asthma. *Lancet* 1983; ii: 1220–3.
- 23 Johnson HG. Cromoglycate and other inhibitors of mediator release. In: Middleton E Jr, Reed CE, Ellis EF, eds. *Allergy—Vol. I, Principles and practice*, 2nd ed. Toronto: C.V. Mosby Company, 1983: 613–32.
- 24 Rhodes EL. Sodium cromoglycate in the treatment of Dermatitis herpetiformis. In: Pepys J, Edwards AM, eds. *The mast cell, its role in health and disease*. Tunbridge Wells: Pitman Medical, 1979: 604–6.
- 25 Mazurek N, Schindler H, Schürholz TH, Pecht I. The cromolyn binding protein constitutes the Ca^{2+} channel of basophils opening upon immunological stimulus. *Proc natl Acad Sci USA* 1984; 81: 6841–5.
- 26 Hogg N, Ross GD, Jones DB, Slusarenko M, Walport MJ, Lachmann PJ. Identification of an anti-monocyte monoclonal antibody that is specific for membrane receptors type one (CR1). *Eur J Immunol* 1984; 14: 236–43.
- 27 Anwar ARE, Kay AB. Enhancement of human eosinophil complement receptors by pharmacological mediators. *J Immunol* 1978; 121: 1245–50.
- 28 Anwar ARE, McKean JR, Smithers SR, Kay AB. Human eosinophil- and neutrophil-mediated killing of schistosomula of *Schistosoma mansoni in vitro*. I. Enhancement of complement-dependent damage by mast cell-derived mediators and formyl methionyl peptides. *J Immunol* 1980; 124: 1122–9.
- 29 Moqbel R, Sass-Kuhn SP, Goetzel EJ, Kay AB. Enhancement of neutrophil- and eosinophil-mediated complement-dependent killing of schistosomula of *Schistosoma mansoni in vitro* by leukotriene B_4 . *Clin Exp Immunol* 1983; 52: 519–27.
- 30 Moqbel R, Nagy L, Lee TH, Sass-Kuhn S, Kay AB. Effect of lipoxygenase products of arachidonic acid

- on complement receptor enhancement and killing of *Schistosoma mansoni*, *in vitro*. In: Piper PJ, ed. Leukotrienes and Other Lipoxygenase Products. Chichester: Research Studies Press, 1983: 241-7.
- 31 MacDonald AJ, Moqbel R, Kay AB. Mediators from IgE-dependent reactions that enhance eosinophil- and neutrophil-mediated complement-dependent killing of schistosomula of *S. mansoni*. *J Allergy Clin Immunol* 1985; 75: 116 (abstr.).
- 32 Capron M, Spiegelberg HL, Prin L, Bennich H, Butterworth AE, Pierce RJ, Ouaisi MA, Capron A. Role of IgE receptors in effector function of human eosinophils. *J Immunol* 1984; 132: 462-8.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.