Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol

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1 The ability of the anti-allergic drugs, sodium cromoglycate (SCG), lodoxamide, traxanox, RU31156 and the β-adrenoceptor agonist salbutamol to inhibit IgE-dependent histamine and prostaglandin D₂ (PGD₂) release was assessed using human dispersed lung mast cells.
2 The anti-allergic drugs were weak inhibitors of histamine release, high concentrations (100–1000 μM) producing < 35% inhibition. Salbutamol produced 39% inhibition at 10 μM.
3 The efficacy of both SCG and salbutamol was inversely related to the concentration of anti-IgE used for challenge and to the degree of histamine release.
4 Rapid tachyphylaxis was observed with all anti-allergic drugs but not with salbutamol.
5 Cross-tachyphylaxis was observed between SCG and the other anti-allergic drugs, suggesting a common mechanism of action. No cross-tachyphylaxis was observed between SCG and salbutamol.
6 SCG was significantly (P < 0.001) more effective in inhibiting PGD₂ than it was histamine release. Preferential inhibition of PGD₂ compared with histamine release was less marked (P < 0.05) with salbutamol and not significant with the other anti-allergic drugs.
7 Mast cells dispersed by enzymatic digestion of human lung released more histamine on immunological challenge than mechanically dispersed cells obtained by fine chopping of tissue. Enzyme treatment of mechanically dispersed cells removed this difference. Enzymatically and mechanically dispersed cells responded similarly to the inhibitory effects of SCG and salbutamol.
8 Our results suggest that salbutamol is a more effective inhibitor of mediator release from human lung mast cells than anti-allergic drugs. However, with the low levels of mediator release achieved during an allergic reaction in man in vivo, both salbutamol and SCG are likely to be effective inhibitors of both preformed and newly generated mediators.

Introduction

The ability of sodium cromoglycate (SCG) and β-adrenoceptor agonists to prevent the immediate bronchoconstrictor response to inhaled allergen is well established. The primary mechanism for SCG has been suggested to be inhibition of the release of bronchoconstrictor mediators from broncho-pulmonary mast cells (Cox, 1967). In support of this mechanism, two studies in vivo have shown some inhibition by SCG of increased circulating histamine and neutrophil chemotactic activity (NCA) following antigen challenge of mild asthmatics (Atkins et al., 1978; Howarth et al., 1985). Although β-adrenoceptor agonists such as salbutamol are considered to relieve bronchoconstriction primarily by relaxing bronchial smooth muscle, these drugs also reduce increases in circulating histamine levels after antigen challenge (Martin et al., 1980; Howarth et al., 1985).

Satisfactory models to define the actions of drugs on mast cell mediators have been hampered by the heterogeneity of this cell both between and within species. In rat connective tissue mast cells from the peritoneum in vitro (Cox, 1967) and in the skin in vivo (Goose & Blair, 1969) SCG is an effective inhibitor of mediator release. However, rapid tachyphylaxis develops to its actions (Sung et al., 1977a,b) and cross-tachyphylaxis with related drugs is taken as an indication of them having the same mechanism of action as...
SCG (Marshall et al., 1976). In contrast, rat mucosal mast cells isolated from the lamina propria of the intestine are refractory to the inhibitory effects of SCG (Pearce et al., 1982). In the guinea-pig, IgE-mediated anaphylaxis is partially inhibited by SCG whereas IgG-mediated reactions are not (Taylor & Roitt, 1973; Carney, 1976). In the mouse, SCG is ineffective regardless of the antibody mediating the reaction (Miller, 1976). Histamine releasing cells of man, like those of the rat, show heterogeneity with respect to the action of SCG. Human basophil leucocytes (Church, 1982) and skin mast cells (Ting et al., 1983; Clegg et al., 1985) are refractory to its inhibitory effects. Immunological histamine release from human lung fragments is partially inhibited by SCG (Butchers et al., 1979; Church & Young, 1983), but the variability of this model makes quantification of responses difficult (Young & Church, 1983). Studies using bronchoalveolar lavage (BAL) mast cells suggest SCG to be active in inhibiting histamine release (Flinth et al., 1985; Leung et al., 1985).

A different profile of activity is seen with β-adrenoceptor agonists. In rat mast cells they are usually inactive against histamine release despite their ability to raise intracellular levels of adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Johnson & Moran, 1970; Marquardt & Wasserman, 1982). In human and guinea-pig lung fragments, β-adrenoceptor agonists are potent inhibitors of histamine release (Assem & Richter, 1971; Butchers et al., 1979; Peters et al., 1982; Church & Young, 1983) and in purified human lung mast cells fenoterol is an effective inhibitor of IgE-dependent histamine release at concentrations below 1 μM (Peters et al., 1982). In contrast, β-adrenoceptor agonists inhibit histamine release from human basophil leucocytes only when they are challenged under unphysiological conditions (Lichtenstein & De Bernardo, 1971).

In this paper we describe the effects of SCG, three SCG-like drugs, lodoxamide, traxanox and RU31156, and the β-adrenoceptor agonist, salbutamol, on IgE-dependent histamine and prostaglandin D₂ (PGD₂) release from human dispersed lung mast cells. With the possibility that dispersion of lung tissue by mechanical and enzymatic methods may preferentially disperse different subpopulations, we have compared the effects of SCG and salbutamol on cells dispersed by both methods.

**Methods**

**Dispersion of human lung cells**

Human lung obtained as fresh surgical specimens within one hour of resection, was dissected free from major bronchi and blood vessels, chopped finely with scissors and suspended in HBSS. Mechanically dispersed lung cells (MDLC) were obtained by separation from tissue fragments by sequential sieving through 800 and 60 μm nylon filters (Church et al., 1982). Enzymatically dispersed lung cells (EDLC), used for the majority of the experiments, were obtained by digestion of the tissue fragments for 30 min at 37°C with pronase, 2 mg ml⁻¹, and chymopapain, 0.5 mg ml⁻¹ (Church et al., 1982). The isolated cells were separated by sieving as described above and the remaining tissue redigested for another 30 min. The cells from the two digests were pooled, washed and resuspended in HBSS. Mast cell numbers were assessed by counting in a Neubauer haemocytometer after metachromatic staining with Kimura stain (Kimura et al., 1973).

**Histamine release experiments and assay**

Immunological histamine release was assessed in duplicate aliquots of 2–4 x 10⁴ mast cells incubated for 15 min with the appropriate concentration of anti-IgE. Reactions were terminated by addition of 0.5 ml ice-cold calcium-free HBSS and centrifugation at 250 g for 10 min at 4°C. The supernatant was acidified to 5% trichloroacetic acid (TCA) and histamine measured spectrofluorimetrically (Church et al., 1982). Spontaneous release was estimated in duplicate tubes to which no anti-IgE had been added. Total histamine was measured in parallel duplicate tubes by disintegrating the cells in 5% TCA. Net histamine release by anti-IgE is expressed as a percentage of total histamine and corrected for spontaneous release.

In time-course studies, 0.02 ml of anti-IgE was added to reaction tubes and warmed to 37°C. At zero time, prewarmed cell suspensions were added to drug solutions or HBSS (control). After the requisite time, 0.48 ml of cell suspension was removed into the reaction tubes to initiate histamine release. Release reactions were stopped 15 min later.

In concentration-response studies, 0.05 ml of drug or buffer and 0.05 ml anti-IgE were added simultaneously to 0.4 ml cell replicates containing 2–4 x 10⁴ mast cells. Release reactions were allowed to proceed for 15 min before termination.

In cross-tachyphylaxis experiments, 0.20 ml of cell suspensions containing 2–4 x 10⁴ mast cells were incubated for 30 min at 37°C with 0.20 ml of 2000 μM SCG (final concentration 1000 μM) or HBSS. After this time, 0.1 ml of anti-IgE plus drug or HBSS was added and the release reaction allowed to proceed for 15 min.

**Prostaglandin D₂ release and assay**

Duplicate 0.4 ml cell replicates containing 4–8 x 10⁴ mast cells were warmed to 37°C before simultaneous
addition of 0.05 ml each of drug or HBSS and anti-IgE. Release reactions were allowed to proceed for 15 min. Because of the degradation of PGD₂ by protein (Fitzpatrick & Wynalda, 1983), FCS was omitted from the buffer in these experiments. PGD₂ was measured in duplicate 0.1 ml aliquots of cell supernatant or diluted cell supernatant to which 0.1 ml of [³H]-PGD₂ (10,000 d.p.m.) had been added followed by 0.1 ml of a 1/100 dilution of anti-PGD₂ serum. After incubation overnight at 4°C, 0.2 ml of dextran coated charcoal was added and the tubes centrifuged at 1500 g for 15 min at 4°C. The supernatant was removed and ³H counted by liquid scintillation spectrometry. The limit of detection of PGD₂ in this assay was 10 pg per tube.

**Materials**

Sodium cromoglycate (SCG) was donated by Fisons Pharmaceuticals, U.K., lodoxamide by the Upjohn Co, U.S.A., traxanox by Yoshitomi Pharmaceutical Industries Ltd, Japan, RU31156 (7-(s-methylsulphonimidoyl)-5-(n-hexyl)-xanthen-9-one-2-carboxylic acid tris-(hydroxymethyl)-aminoethane salt) by Roussel Labs., U.K. and salbutamol by Glaxo Group Research, U.K. Goat anti-human IgE was heat inactivated by incubation at 56°C for 1 h (Church et al., 1982). Deoxyribonuclease (DNase, bovine pancreas), chymopapain (papaya latex), pronase (Streptomyces griseus) and N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES) were purchased from Sigma, U.K. Foetal calf serum (FCS) was obtained from GIBCO, Scotland. Rabbit anti-prostaglandin D₂ serum was purchased from Dr L. Levine, Brandeis University, Waltham, M.A., U.S.A. and [5,6,8,9,12,14,15, (n)-³H]-PGD₂ (100 Ci mmol⁻¹) from Amersham International, U.K. All other chemicals were purchased from BDH, Poole, U.K. The composition of HEPES buffered salt solution (HBSS) was (in mM): sodium chloride 137, glucose 5.5, sodium dihydrogen phosphate 0.4, potassium chloride 2.7, magnesium chloride 0.5, calcium chloride 0.9 and HEPES 10. The pH was adjusted to 7.4 with 2M sodium hydroxide and 2.0% FCS added.

**Results**

**Time-course studies**

As rapid tachyphylaxis is a consistent feature of the action of anti-allergic drugs in rat mast cells (Sung et al., 1977a,b), initial experiments were performed to define the optimal preincubation time for human lung mast cells with inhibitory drugs. The results (Figure 1) show that in seven experiments all drugs tested were maximally active when added simultaneously with anti-IgE (1%) challenge. With SCG, activity waned as the preincubation time of drug with cells before challenge was increased up to 15 min. Similar effects were observed with lodoxamide, traxanox and RU31156. With longer preincubation periods, there was a tendency for the anti-allergic drugs to 'regain' their efficacy, a phenomenon more marked with traxanox and RU31156.

Salbutamol was also maximally active when added to the cells simultaneously with anti-IgE. Although its activity also waned with extended preincubation periods (Figure 1), this was gradual and did not have the characteristics observed with the anti-allergic drugs.

**Concentration-response studies**

Figure 2 shows the results of concentration-response studies when drugs were added to the dispersed lung cells simultaneously with anti-IgE (1%) challenge. High concentrations of anti-allergic drugs were required to observe significant inhibition. SCG produced only 24.6 ± 4.7% inhibition of histamine release at 1000 μM. Lodoxamide was a little more active, producing 33.2 ± 6.2% inhibition at 1000 μM.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Time course for inhibition of IgE-dependent histamine release from human enzymatically dispersed lung cells by (●) sodium cromoglycate (100 μM), (▲) lodoxamide (100 μM), (▼) traxanox (100 μM), (■) RU31156 (100 μM) and (○) salbutamol (100 μM). Each result is the mean of observations in seven experiments in which net histamine release at each time point (range 18.5–19.8%) was corrected for spontaneous release assessed at that time (range 5.4 ± 0.8% to 7.7 ± 1.1%). Standard errors, which have been omitted from the graph for clarity, ranged between 1.0 and 7.3 (mean 4.6) % inhibition of histamine release.
Limited aqueous solubility prevented traxanox and RU31156 being tested at this concentration, but inhibitions of 23.5 ± 3.5% and 30.8 ± 6.7% at 100 µM suggested both of them to be marginally more active than SCG. Salbutamol was markedly more potent than the other drugs, producing 38.7 ± 6.3% inhibition of histamine release at 10 µM.

**Anti-IgE concentration**

The effects of SCG and salbutamol were assessed on histamine release induced by three concentrations of anti-IgE, 0.3%, 1% and 10% which released 16.1 ± 5.0%, 20.8 ± 5.0% and 27.9 ± 5.5% histamine, respectively. The results (Figure 3) showed that with both drugs the degree of inhibition was inversely related to the concentration of anti-IgE used for challenge ($r = 0.95$, $P < 0.001$, $n = 8$) and to the net control histamine release ($r = 0.98$, $P < 0.001$, $n = 8$). In these experiments, significant concentration-related effects of SCG were only observed at the lowest concentration of anti-IgE, 0.3%, a maximum inhibition of 28.7 ± 5.7% being observed at 1000 µM. In contrast, salbutamol produced concentration-related inhibitions at all anti-IgE concentrations, maximum inhibitions being 53.0 ± 8.1%, 38.2 ± 6.1% and
17.7 ± 4.8% at 10 µM at 0.3, 1 and 3% anti-IgE, respectively.

**Effect on prostaglandin D₂ release**

The possibility that drugs could preferentially inhibit the release of a newly generated mediator, PGD₂, was examined in four experiments (Figure 4). SCG at 1000 µM was significantly (P < 0.001) more effective in suppressing PGD₂ release, 84.8 ± 6.1% inhibition, than histamine release, 24.8 ± 3.8% inhibition. With lodoxamide, traxanox and RU31156, the inhibitions of histamine and PGD₂ release were not statistically different. Salbutamol also showed a preferential inhibition of PGD₂ generation (P < 0.05), but this was less marked than with SCG. With salbutamol, histamine release was inhibited by 31.6 ± 2.2% and PGD₂ by 65.0 ± 10.2%.

**Cross-tachyphylaxis**

In rat mast cell experiments, the demonstration of cross-tachyphylaxis between SCG-like compounds is taken as an indicator that they have the same mechanism of action (Marshall et al., 1976). We investigated cross-tachyphylaxis between SCG and the other drugs used in this study in three experiments (Table 1). Cells were preincubated for 30 min at 37°C with either 1000 µM SCG or HBSS alone (control) before simultaneous addition of the second drug and 1% anti-IgE. Preincubation with SCG completely blocked the subsequent effect of 100 µM SCG. That the effect of 1000 µg SCG was only partially reversed shows that tachyphylaxis may be partially overcome by using a larger concentration of SCG at the time of challenge. Lodoxamide, traxanox and RU31156 all showed significant (P < 0.05) cross-tachyphylaxis suggesting a common mechanism of action in human lung mast cells. In contrast, salbutamol showed no cross-tachyphylaxis with SCG.

**Table 1** Cross-tachyphylaxis between sodium cromoglycate (SCG) and other drugs which inhibit histamine release from human dispersed lung cells

<table>
<thead>
<tr>
<th>Drug</th>
<th>Inhibition of histamine release (%)</th>
<th>Reduction in efficacy (%)</th>
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<tbody>
<tr>
<td>SCG 100 µM</td>
<td>16.0 ± 4.7</td>
<td>100*</td>
</tr>
<tr>
<td>SCG 1000 µM</td>
<td>27.9 ± 3.7</td>
<td>46</td>
</tr>
<tr>
<td>Lodoxamide 100 µM</td>
<td>29.9 ± 5.2</td>
<td>83*</td>
</tr>
<tr>
<td>Traxanox 100 µM</td>
<td>25.0 ± 7.3</td>
<td>52*</td>
</tr>
<tr>
<td>RU31156 100 µM</td>
<td>32.1 ± 0.7</td>
<td>54*</td>
</tr>
<tr>
<td>Salbutamol 10 µM</td>
<td>38.7 ± 5.2</td>
<td>4</td>
</tr>
</tbody>
</table>

Each result is the mean ± s.e.mean of three experiments in which cells were preincubated for 30 min at 37°C with HBSS (control) or SCG, 1000 µM, before the simultaneous addition of the second drug and anti-IgE, 1%. Mean net control histamine release was 14.4 ± 3.2% and spontaneous release 5.2 ± 1.6%. * Indicates significant (P < 0.05) cross-tachyphylaxis.
Figure 5  Net histamine release from human dispersed lung mast cells induced by anti-IgE. (a) Histamine release from mechanically (○) and enzymatically (△) dispersed cells. Each result is the mean of four experiments in which spontaneous histamine releases were 12.7 ± 1.9% and 14.2 ± 1.1%, respectively. (b) Histamine release from mechanically dispersed cells (○) and mechanically dispersed cells exposed to pronase and chymopapain (●). Each result is the mean of four experiments in which spontaneous histamine releases were 17.3 ± 3.4% and 16.0 ± 4.8%, respectively. Vertical lines indicate s.e.mean.

whether mechanical and enzymatic dispersion methods yielded different subpopulations of mast cells, the two methods were compared. In four experiments, both mechanically (MDLC) and enzymatically (EDLC) dispersed lung cells prepared from the same lung samples showed a concentration-related release of histamine following challenge with anti-IgE (Figure 5a). Multivariate analysis revealed that EDLC released significantly (P < 0.001) more histamine than did MDLC. That enzyme treatment was more likely to be revealing additional functional IgE-receptors rather than dispersing a different subpopulation of mast cells was suggested by four further experiments in which treatment of MDLC with pronase and chymopapain significantly (P < 0.005) enhanced their ability to release histamine upon incubation with anti-IgE (Figure 5b).  

The effects of SCG and salbutamol on IgE-dependent histamine release from MDLC and EDLC were examined in three experiments (Table 2). The results show no significant differences between the effects of drugs on the two cell populations.

Table 2  Effect of sodium cromoglycate (SCG) and salbutamol on human mechanically (MDLC) and enzymatically (EDLC) dispersed lung cells

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (μM)</th>
<th>MDLC</th>
<th>EDLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCG</td>
<td>10</td>
<td>13.4 ± 8.6</td>
<td>18.2 ± 7.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20.8 ± 6.0</td>
<td>19.8 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>32.4 ± 11.3</td>
<td>25.9 ± 6.5</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>0.1</td>
<td>50.8 ± 9.1</td>
<td>49.5 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>54.9 ± 6.0</td>
<td>59.8 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>66.2 ± 5.9</td>
<td>64.3 ± 2.4</td>
</tr>
</tbody>
</table>

Each result is the mean ± s.e.mean of three experiments in which drugs were added to cells simultaneously with anti-IgE, 0.3%, challenge. Mean control net histamine release for MDLC was 14.0 ± 5.0% and spontaneous release 12.8 ± 2.1%. Corresponding values for EDLC were 18.2 ± 9.2% and 12.0 ± 3.1%, respectively. There were no significant differences between MDLC and EDLC.
Discussion

Experiments using enzymatically dispersed human lung mast cells have shown SCG to be a weak inhibitor of histamine release, high concentrations being required for significant activity. These results confirm observations using human lung fragments (Butchers et al., 1979; Church & Young, 1983) but contrast with results obtained in rat mast cells (Goose & Blair, 1969; Sung et al., 1977a). In comparison, the β-adrenoceptor agonist, salbutamol, was more effective, producing a greater inhibition of histamine release at lower concentrations, again confirming previous reports using lung fragments (Butchers et al., 1979; Church & Young, 1983) and consistent with the effects of fenoterol in purified lung mast cells (Peters et al., 1982). Comparison of the concentrations required to produce 25% inhibition of histamine in all experiments where both drugs were used, suggested salbutamol to be between 2000 and 30,000 times more potent than SCG.

Comparisons of the effects of SCG and related drugs in human and rat mast cell preparations show both similarities and differences. The most obvious similarity is the development of rapid tachyphylaxis, a phenomenon repeatedly found for rat mast cells (Sung et al., 1977a,b) which is suggested to be associated with its mechanism of action, i.e. the transient appearance of a phosphorylated 78,000 dalton protein which inhibits mediator release (Theoharides et al., 1980; Wells & Mann, 1983). A second similarity is the presence of cross-tachyphylaxis between SCG and related drugs (Marshall et al., 1976). Thus, the observation that preincubation of cells with SCG significantly reduced the subsequent effects of loxodamide, traxanox and RU31156 suggests that they inhibit histamine release from human lung mast cells by a similar mechanism. This mechanism is obviously related to that of salbutamol which showed no cross-tachyphylaxis with SCG. Differences between the effects of SCG and related drugs in rat and human lung mast cells are the high concentrations required and the weak inhibitory effect in human cells (Goose & Blair, 1969). Furthermore, SCG, lodoxamide, traxanox and RU31156 have similar potencies in human lung mast cells whereas in the rat PCA test (which is indicative of activity in rat connective tissue mast cells) their potencies are widely differing being (based on SCG = 1); lodoxamide 2500 (Johnson et al., 1978), traxanox 8 (Goto et al., 1979) and RU31156 260 (Miller & James, 1978).

Immunological activation of human lung mast cells not only releases histamine but also leads to the synthesis and release of PGD₂ (Holgate et al., 1984) and sulphidopeptide leukotrienes (MacGlashan et al., 1982). Although leukotrienes may also be released from other cells within the dispersed samples (Damon et al., 1983), the mast cell is the only source of PGD₂ (Holgate et al., 1984). Our observation that SCG inhibits the release of PGD₂ more effectively than that of histamine, therefore, reflects a differential inhibition of mediator release within the same cell population. The differences between inhibition of histamine and PGD₂ liberation were not significantly different with the other anti-allergic drugs tested. Even though PGD₂ is a potent bronchoconstrictor prostaglandin in man (Hardy et al., 1984), it is not clear how inhibition of PGD₂ production relates to its clinical efficacy. However, it is noteworthy that while both SCG and lodoxamide inhibit the early asthmatic reaction to bronchial provocation (Cox & Altounyan, 1970; Watt et al., 1980), lodoxamide has little activity against clinical asthma (Mann et al., 1985).

The inhibitory effects of salbutamol and SCG were inversely related to the intensity of immunological stimulation and, consequently, to histamine release. With salbutamol, the slopes of the concentration-inhibition curves and the maximum inhibition of histamine release were inversely related to the strength of immunological stimulation. However, the maximally effective concentration, 1 to 10 μM, remained constant. These results are consistent with the findings of Tung & Lichtenstein (1981) that the inhibitory capacity of agents which elevate intracellular cyclic AMP is more pronounced at low levels of histamine release. The observation of a similar relationship with SCG suggests that these characteristics also apply to agents utilizing cyclic GMP as an intracellular messenger (Wells & Mann, 1983). As, in comparison to in vitro tests, the level of immunological stimulation and consequential histamine release during allergic reactions in man in vivo are low (Howarth et al., 1985), it is likely that both salbutamol and SCG would be effective inhibitors of mast cells mediator release in the clinical situation.

Recent findings suggest that SCG is more effective in inhibiting histamine release in mast cells removed from the lung by bronchoalveolar lavage (BAL) than it is in cells dispersed from lung tissue (Flint et al., 1985). It was considered possible that mechanical dispersion of human lung fragments by fine chopping with scissors may preferentially disperse mast cells loosely associated with the mucosa of the smaller bronchi as opposed to those deeper in the connective tissue, which are yielded by enzymatic dispersion techniques. Although initial experiments revealed that immunologically activated enzymatically dispersed cells released a significantly greater quantity of histamine than mechanically dispersed cells, this is likely to result from an effect of the enzymes on the cell surface rather than dispersion of subpopulations of cells. The similarity of inhibition of histamine release by SCG and salbutamol would support this suggestion. The apparent differences between the effects of SCG on
dispersed and BAL cells cannot be readily explained by the dispersion or collection methods and may, therefore, reflect either the predominance of sub-populations of mast cells or cells in a different state of activation.

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