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Activation of human lung mast cells by monomeric immunoglobulin E

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ABSTRACT: The mechanism of chronic mast cell activation in asthma is unclear. Monomeric immunoglobulin (Ig)E in the absence of allergen induces mediator release from rodent mast cells, indicating a possible role for IgE in the continued activation of mast cells within the asthmatic bronchial mucosa. In this study it was investigated whether monomeric IgE induces Ca^{2+} influx and mediator release from human lung mast cells (HLMC).

Purified HLMC were cultured for 4 weeks and then exposed to monomeric human myeloma IgE. Ratiometric Ca^{2+} imaging was performed on single fura-2-loaded cells. Histamine release was measured by radioenzymatic assay; leukotriene C_4 (LTC_4) and interleukin (IL)-8 were measured by ELISA.

At concentrations experienced *in vivo*, monomeric IgE induced dose-dependent histamine release, LTC_4 production and IL-8 synthesis. This was associated with a rise in cytosolic free Ca^{2+}. Enhanced histamine release was still evident 1 week after initial exposure to IgE suggesting that continued exposure maintains enhanced secretion.

Monomeric immunoglobulin E alone activates cultured human lung mast cells initiating Ca^{2+} influx, degranulation, arachidonic acid metabolism and cytokine synthesis. These findings support the hypothesis that immunoglobulin E loading of mast cells within the asthmatic airway contributes to the disordered airway physiology of this disease.

KEYWORDS: Asthma, calcium, human, mast cell, mediators, monomeric immunoglobulin E

Mast cells play a central role in the immunopathology of asthma through the sustained secretion of a plethora of proinflammatory mediators, cytokines and proteases (reviewed in [1]). They infiltrate key structures in asthmatic airways, such as the airway smooth muscle (ASM) [2] and airway mucous glands [3], events that are likely to be critical for the targeting of mediators to their intended site of action.

It is often assumed that chronic mast cell activation in asthma is driven by exposure of the airways to inhaled aeroallergens, resulting in cross-linking of allergen-specific immunoglobulin (Ig)E bound to the mast cell high affinity IgE receptor FcεRI. However, allergen avoidance usually has only a minor effect on the state of established asthma, which appears to become ''self-perpetuating'' [4]. Mast cell hypersecretion in on-going asthma may therefore arise from alternative stimuli, for example by proteases [5], adenosine [6], and by cytokines, such as stem cell factor (SCF) [7]. Of great interest, monomeric IgE alone can induce mediator release from the rat mast cell line RBL-2H3 and mouse bone marrow-derived mast cells (BMMC) in the absence of IgE cross-linking by antigen [8, 9]. In addition, IgE prolongs rodent mast cell survival, in part through the autocrine release of survival-enhancing cytokines, such as interleukin (IL)-6 [8]. For reasons that are unknown, the extent of these responses depends on the source of the IgE. Thus, some types of IgE induce both histamine and cytokine synthesis, while others induce the release of cytokines only [8]. These observations are particularly interesting because in humans there is a reproducible correlation between total serum IgE concentration, bronchial hyperresponsiveness and asthma [10–13]. Thus, it is attractive to hypothesise that in asthma, heightened mast cell activation within the airways arises, at least in part, from the increased binding of IgE to FcεRI.

The effects of monomeric IgE on human lung mast cell (HLMC) function are unknown. There are important functional differences between rodent and human mast cells, which are typified by the marked differences in ion channel activity the current authors have recently demonstrated [14]. Therefore, ultimately, studies must be performed on human cells and from the tissue of interest. In this study, the present authors have

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taken the first step in testing their hypothesis. They demonstrate that IgE, in the absence of cross-linking by allergen, activates cultured HLMC resulting in Ca^{2+} influx and the release of histamine, leukotriene C_4(LTC_4) and IL-8. This has important implications for the pathogenesis of asthma.

METHODS

HLMC purification and culture

All human subjects gave written informed consent and the study was approved by the Leicestershire Research Ethics Committee, UK. HLMC were dispersed and purified from macroscopically normal lung obtained within 1 h of resection for lung cancer using immunoaffinity magnetic selection as described previously [15]. The final HLMC purity was >98% with cell viability >97% (monitored by exclusion of trypan blue). Following isolation, HLMC were cultured in Dulbecco's modified essential medium (DMEM)/Glutamax/HEPES containing 10% FBS, 1% MEM nonessential amino acids (all from Life Technologies, Paisley, UK), 1% antibiotic/antimycotic solution (Sigma, Poole, UK), 100 ng·mL^{-1} recombinant human (rh)SCF, 50 ng·mL^{-1} rhIL-6 and 10 ng·mL^{-1} rhIL-10 (R&D, Abington, UK) at 37℃ in a humidified incubator flushed with 5% CO_2 for a minimum of 4 weeks prior to experiments [16]. Half of the medium was changed every 7 days.

Ca^{2+} imaging

Changes in cytosolic-free Ca^{2+} ($[Ca^{2+}]_i$) were monitored fluorometrically by use of the Ca^{2+}-sensitive probe Fura-2 as described previously [16]. Data acquisition occurred at a rate of one dual-wavelength image every 6 s as the 340/380 nM ratio. This was converted to $[Ca^{2+}]_i$ using a commercially available calibration kit (Molecular probes). IgE was added to the dish as required.

HLMC activation for mediator release

Mast cells (1×10^4) were warmed to 37℃ in triplicate in 50 µL of DMEM and 50 µL monomeric human myeloma IgE (Calbiochem-Novabiochem, Nottingham, UK), or human IgG (Sigma, Poole, Dorset), at 2× the final concentration was then added. After incubation for 30 min or 24 h at 37℃, the cells were centrifuged at 250×*g* for 4 min, and the supernatant decanted. Control cell pellets were lysed in sterile deionised water for measurement of total histamine content.

To investigate whether there were any aggregates of IgE present in the IgE preparation, freshly constituted IgE was centrifuged at 14000×*g* for 20 min, the supernatant removed and any aggregates resuspended in PBS. A dose-response of centrifuged IgE, any aggregated IgE and the normal IgE was then run in parallel.

Mediator assays

Histamine was measured by radioenzymatic assay, as described previously [15]. LTC_4 (Cayman Chemical Co., Ann Arbor, MI, USA) and IL-8 (BD Biosciences, San Diego, CA, USA) were measured by ELISA according to the manufacturer's instructions.

Data presentation and statistical analysis

Data is expressed as the mean±SEM unless otherwise stated. Histamine, LTC_4 and IL-8 are expressed in ng·10^{-6} cells released into supernatants. Differences between groups of data were evaluated using paired or unpaired (two tailed) t-test as appropriate. A p-value of <0.05 was considered statistically significant.

RESULTS

Monomeric IgE induces the release of histamine, LTC_4 and IL-8 from HLMC

Since SCF is an essential growth factor for human mast cells, withdrawal of which induces apoptosis, and which is present even in normal tissues but elevated in asthmatic airways [17], the effect of monomeric IgE on HLMC incubated in their normal SCF-supplemented growth medium was first examined. The early release (30 min) of pre-formed granule-derived histamine and the newly synthesised product of arachidonic acid metabolism LTC_4, in addition to the late (24 h) release of the chemokine IL-8, was studied. Interestingly, the current authors found that HLMC from several separate donors released histamine and LTC_4 in a dose-dependent manner when incubated with IgE for 30 min in the presence of SCF (fig. 1 a and b). Thus, control histamine release was 258±26 ng·10^{-6} cells compared with 583±99 ng·10^{-6} cells with the addition of 3 µg·mL^{-1} IgE, giving a net monomeric IgE-dependent histamine release of 325±88 ng·10^{-6} cells (12.8% of cell total) ($p=0.008$, $n=8$; fig. 1 a). There was a parallel increase in the release of LTC_4 with net monomeric IgE-dependent release of 4.3±1.2 ng·10^{-6} cells at a concentration of 3 µg·mL^{-1} IgE ($p=0.038$, $n=4$; fig. 1 b). IL-8 concentrations were measured after 24 h in cells activated with 3 µg·mL^{-1} IgE and were significantly elevated compared with control (control 2.77±0.51 ng·10^{-6} cells, IgE 7.33±0.19 ng·10^{-6} cells, $p=0.003$, $n=4$; fig. 1 c). To see if this effect was IgE specific, a parallel test was run using IgG and there was found to be no release of histamine in these cells (299±27 ng·10^{-6} cells in the control cells, compared with 273±42 ng·10^{-6} cells with the addition of 3 µg·mL^{-1} IgG, $p=0.37$, $n=4$).

In vivo, serum IgE concentrations remain fairly stable, so it was important to assess whether the effects of monomeric IgE were sustained. In three further donors, HLMC were cultured with or without 3 µg·mL^{-1} IgE for 7 days in their normal SCF-supplemented growth medium before washing to remove accumulated histamine and resuspending in medium±IgE. Histamine release was 125±27 ng·10^{-6} cells without IgE compared with 1033±480 ng·10^{-6} cells in the presence of 3 µg·mL^{-1} IgE, giving a net histamine release of 909±288 ng·10^{-6} cells (24.6% of cell total) ($n=3$, $p=0.05$). This suggests that IgE signalling is maintained when FcεRI are occupied.

Although less physiological, most studies investigating human mast cell histamine release have done so in the absence of SCF. When HLMC were activated with monomeric IgE in the absence of SCF this also resulted in acute histamine release in 12 of 14 donors, although this was significantly lower than in the experiments in the presence of SCF. Thus, with 3 µg·mL^{-1} monomeric IgE, histamine release increased from 162±51 ng·10^{-6} cells to 213±59 ng·10^{-6} cells, giving a net histamine release of 51±10 ng·10^{-6} cells (1.7% of cell total) (all 14 donors, $p=0.0002$). This histamine release was also found to be accompanied by arachidonic acid metabolism even in SCF-deprived cells, exemplified by the release of LTC_4 in the IgE treated cells, rising from 175±28 pg·10^{-6} cells in the control

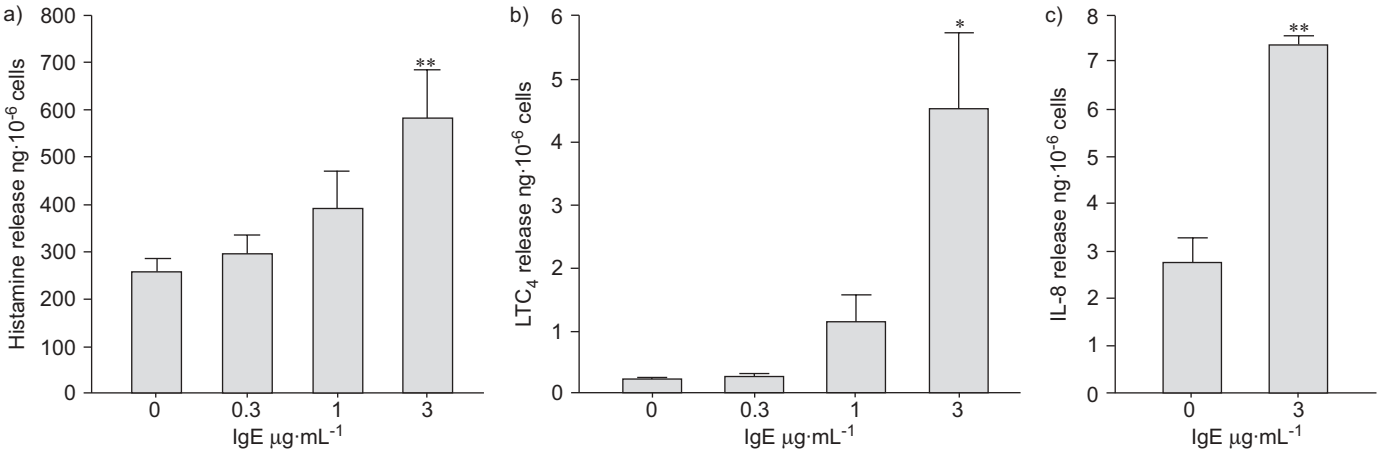

FIGURE 1. The activation of human lung mast cells by monomeric immunoglobulin (Ig)E in the presence of stem cell factor 100 ng·mL^{-1}. IgE alone induces the dose-dependent release of a) the pre-formed granule-derived mediator histamine (n=8), b) the *de novo* synthesised arachidonic acid metabolite leukotriene C_4 (LTC_4; n=4), and c) newly synthesised interleukin (IL)-8 (n=4). All values are mean±SEM. *: $p<0.05$; **: $p<0.01$.

cells to 348±76 pg·10^{-6} in the 3 μg·mL^{-1} IgE-treated cells ($p=0.046$, n=10) (net release of 172±75 pg·10^{-6} cells).

The activation of mast cells by IgE alone often raises the question of whether the IgE used is truly monomeric. It is conceivable that the effects reported are caused by low level aggregates of IgE in the preparations, which when added to the cells cross-link FcεRI, thus mimicking allergen exposure. To address this, freshly constituted IgE was centrifuged at 14000×*g* for 20 min, the supernatant removed and any potential aggregates resuspended in PBS. A dose–response of centrifuged IgE was then run, with any aggregated IgE and the normal IgE in parallel, and there was found to be no significant difference between the centrifuged and normal IgE (net histamine release of 51±7 ng·10^{-6} cells and 57±8 ng·10^{-6} cells, respectively with 3 μg·mL^{-1} IgE). In addition, if any aggregates were in the precipitate, they did not elicit any response in the cells (net histamine release 5±2 ng·10^{-6} cells), which is consistent with the previous observations by KALESNIKOFF *et al*. [18].

Monomeric IgE increases cytosolic-free Ca^{2+}

Next to be investigated was whether monomeric IgE-dependent histamine release from HLMC was associated with a rise in $[Ca^{2+}]_i$. It was found that in the absence of SCF, monomeric IgE induced a significant increase in $[Ca^{2+}]_i$ from a basal level of 277.4±4.7 nM 3 min prior to the addition of IgE to 376.2±8.39 nM 2 min following the addition of 3 μg·mL^{-1} IgE ($p=0.0001$, n=162 cells from four separate donors; fig. 2). The current authors have recently shown that there is a tight correlation in HLMC between change in $[Ca^{2+}]_i$ and the magnitude of anti-IgE-dependent histamine release [19]. From this relationship, the change in $[Ca^{2+}]_i$ in the present study would predict a net histamine release of ~3% of the cell total, which is very close to that actually measured (2.0% excluding nonresponders). The Ca^{2+} plateau was relatively smooth, in keeping with the changes reported in HLMC after anti-IgE-dependent activation, although occasional cells exhibited oscillations similar to those described previously in basophils [20]. This suggests that the signalling pathways activated by monomeric IgE and anti-IgE may be similar, in keeping with the recent conclusions from rodent studies [21].

DISCUSSION

This study makes the novel observation that cultured HLMC release histamine, LTC_4 and IL-8 on acute exposure to monomeric IgE and that this effect is maintained while IgE remains present. The effects are relatively small compared with anti-IgE-dependent activation, particularly in the absence of SCF, but even this magnitude of mediator release may be of great physiological significance where mast cells infiltrate key tissue structures.

Many *in vitro* studies on mast cells have relied on maximally activating the cells with FcεRI cross-linking, leading to anaphylactic degranulation and massive histamine release. However, this level of maximal cell activation is of questionable physiological relevance because it is probably never achieved in humans *in vivo*, except perhaps during anaphylaxis and laboratory allergen challenge. Certainly in chronic on-going asthma, mast cell activation is far less aggressive, with piecemeal degranulation evident on electron microscopy rather than the anaphylactic degranulation that occurs with maximal cell activation [22]. There are also significantly lower concentrations of mediators present in bronchoalveolar lavage fluid in stable asthma compared with post-allergen challenge [23]. Thus, the relatively subtle release of mediators demonstrated with monomeric IgE may be important when there is close approximation of target cells. This is particularly relevant in asthma where there is microlocalisation of mast cells within specific tissue structures, such as the ASM [2]. The observation that HLMC not only degranulate but also metabolise arachidonic acid and synthesise cytokines in response to IgE suggests that several diverse tissue effects will ensue. For example, histamine is a bronchoconstrictor, tryptase is an ASM mitogen [24], cysteinyl leukotrienes are potent bronchoconstrictors and potentiate ASM proliferation following exposure to IL-13 [25], while IL-8 is also a potent smooth muscle mitogen [26]. Furthermore, since concentrations of SCF are elevated in asthmatic airways [17], and SCF is a product of ASM [27], it

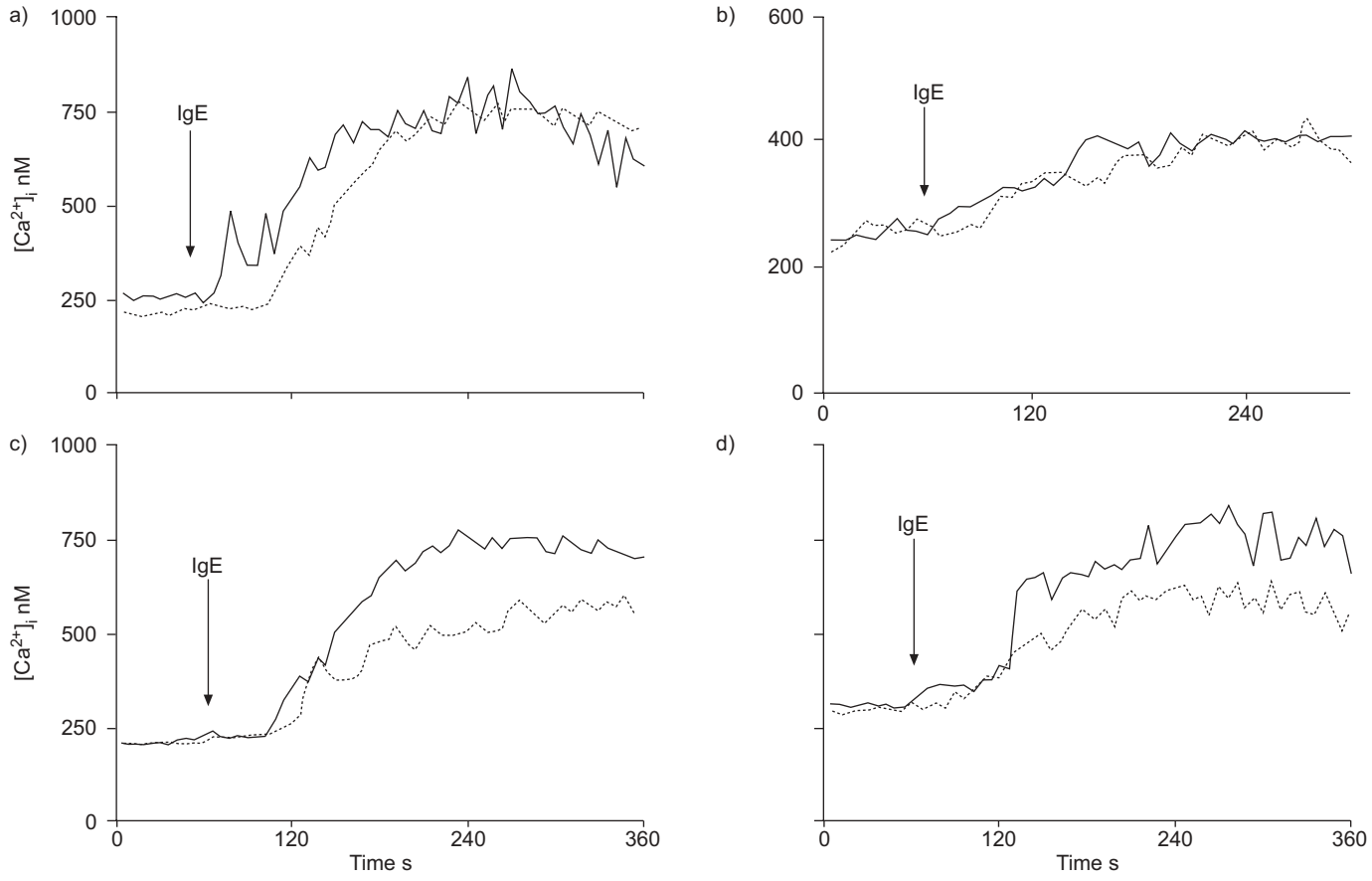

FIGURE 2. Heterogeneity of changes in $[Ca^{2+}]_i$ in human lung mast cells during incubation with 3 μg·mL^{-1} monomeric immunoglobulin (Ig)E. Each panel represents a different donor and each trace an individual cell.

is easy to envisage a positive feedback loop whereby monomeric IgE together with ASM-derived SCF activates mast cells, which in turn activate ASM. Such a pathway of mast cell activation in asthma would help explain the strong correlation evident between total rather than specific serum IgE and the degree of bronchial hyperresponsiveness [10–13].

Serum concentrations of IgE remain relatively constant over time, so it is important that the current authors have shown that 1 week after exposure to IgE, the potentiated HLMC secretory response is maintained. This is consistent with the data from RBL-2H3 cells, showing that cell signalling continues as long as unbound IgE is present. Removal of ''free'' IgE quickly returns the cells to quiescence. Reintroduction of IgE to the sensitised cells promptly recommences signalling, suggesting that bound IgE does not prevent ''free'' IgE signalling [21]. These observations, therefore, provide a further mechanism for the enhanced constitutive mediator release evident in mast cells obtained from asthmatic *versus* normal subjects [28]. It is also of note that the top concentration of IgE studied, 3 μg·mL^{-1}, is equivalent to ~1250 iU·mL^{-1}, well within the range experienced *in vivo*.

In this study lung mast cells that have been maintained in culture for at least 4 weeks have been used, as described recently by the current authors [16]. This was for several reasons. First, freshly isolated mast cells from normal donors are relatively quiescent compared with those from asthmatic subjects, and a large proportion of the cells do not express FcεRI [28, 29]. Those that do express FcεRI are likely to have them occupied by endogenous IgE, which would require stripping at low pH, resulting in cell damage. In contrast, the cultured cells all express FcεRI, and when sensitised with IgE release greater quantities of mediators in response to anti-IgE than freshly isolated cells [16, 19]. More importantly, about half the cells die within the first week of culture, but then proliferate, so that by 4 weeks there are up to five times the starting number [16]. Thus, ⩾90% of the cells at 4 weeks will never have been exposed to IgE, making the experiments viable. Also, since there is a clearly increased expression of SCF in asthmatic compared with normal airways [17], these cells that have been cultured in SCF may also be more representative of the asthmatic phenotype than those from normal subjects.

When examining the effects of IgE on HLMC activation, it is important to ensure that the IgE used is indeed monomeric. IgE preparations were therefore centrifuged and a dose–response of centrifuged IgE, uncentrifuged IgE and any aggregated IgE was ran in parallel and there was found to be no significant difference between the centrifuged and uncentrifuged preparations. This is consistent with the observation by KALESNIKOFF *et al*. [18], that although the IgE used in their experiments (SPE-7 anti-dinitrophenol) contained aggregates,

the aggregates themselves were considerably less effective at eliciting a response than monomeric IgE [18].

Previous work from the mouse and RBL-2H3 cells suggests that the mechanism by which monomeric IgE results in mast cell activation is through enhanced dimerisation of FcεRI [18, 21]. This would suggest that the signalling pathway activated should be similar to that activated by anti-IgE. In terms of Ca^{2+} mobilisation, in previous murine studies IgE alone has been reported to cause Ca^{2+} mobilisation leading to a rise in $[Ca^{2+}]_i$, but which fails to reach the threshold necessary for degranulation to occur. Studies by HUBER *et al*. [30] and KALESNIKOFF *et al*. [18] found that the degranulatory response to IgE alone in wild-type BMMC was not significant despite an increase in $[Ca^{2+}]_i$ and evidence that both β and γ subunits of FcεRI, along with many Erk kinases and SH2-containing inositol 5′-phosphatase, were tyrosine phosphorylated [18]. Another study by OKA *et al*. [9] reports that the addition of monomeric IgE to RBL-2H3 cells induced a significant increase in both $[Ca^{2+}]_i$ and mediator release. However, in the same study, murine BMMC exhibited markedly less (but still significant) degranulation than RBL-2H3 cells, despite the $[Ca^{2+}]_i$ increase being comparable [9]. It has been shown that the Ca^{2+} signals observed in HLMC were similar to those described previously by the current authors and others, following anti-IgE-dependent cell activation [19, 20]. This suggests that the signalling pathways activated by anti-IgE and monomeric IgE share several components, but whether these are identical will require further study.

Interestingly, in contradiction to the current authors' results, the only previous study of monomeric IgE on human mast cell activation used cord-blood-derived mast cells and reported no significant effect on histamine or IL-8 release, although this was in the absence of SCF [31]. It is well recognised that there is marked heterogeneity in mast cell responses both between species and from different tissues within the same species, so the data again highlights the importance of studying cells from the tissue of interest.

In conclusion, the results indicate that monomeric immunoglobulin E alone induces the release of histamine, leukotriene C_4 and interleukin-8 from human lung mast cells. The magnitude of release is less than the massive release that can be obtained with anti-immunoglobulin E or allergen, but this may actually be more physiologically relevant. Since mast cells infiltrate key airways structures, such as the airway smooth muscle in asthma, the diverse array of mediators secreted in response to monomeric immunoglobulin E may have critical consequences for the function of these cells. These findings would help explain the strong correlations observed between total serum immunoglobulin E and the presence of asthma and bronchial hyperresponsiveness, and the ability of anti-immunoglobulin E treatment to ameliorate the disease in contrast to allergen avoidance [32]. The ability of monomeric immunoglobulin E to activate human lung mast cells could, therefore, be central to the chronic symptoms of established asthma.

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