

The role of mast cells and basophils in inflammation

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Summary

Mast cells are positioned in the asthmatic airways so that they are able to respond to the inhaled environment. During active disease, the cells are primed to secrete an array of preformed and newly generated inflammatory mediators including histamine, neutral proteases and heparin sulphate, prostaglandins and cysteinyl leukotrienes as well as an array of cytokines and chemokines that are involved in leucocyte recruitment and activation. These cells are a potent source of mediators in both allergen and exercise-induced asthma and possibly in asthma provoked by other stimuli such as adenosine and inhaled air pollutants. The important role played by mast cells in maintaining airway dysfunction in asthma is underpinned by the efficacy of mediator inhibitors, such as those interfering with the release or action of the leukotrienes, agents that inhibit mast cell activation such as sodium cromoglycate and the recently studied E-20 humanized monoclonal antibody that binds to and removes IgE. The recent discovery of novel inhibitory pathways involving inhibitory motifs (ITIMS) on critical cell surface signalling molecules has opened up new possibilities for preventing mast cell activation. Future research will focus on more effective ways for inhibiting the mast cell's contribution to asthma and understanding what role this unique cell has in the pathogenesis of airway wall remodelling.

Keywords: mast cells, basophils, inflammatory mediators

The mast cell has a long history in being recognized as an important mediator-secreting cell in asthma. Its capacity to release an array of both preformed and newly generated mediators in response to environmental stimuli especially allergen exposure (Table 1). Cross linkage of IgE bound to high affinity receptors on mast cells not only results in the rapid release of autacoid mediators, but also the sustained synthesis and release of cytokines, chemokines and growth factors [1] (Table 2). It is this component that has now placed the mast cell among the important cells that contribute to the ongoing chronic inflammation in asthma. Mast cells are also a rich source of neutral proteases, specifically tryptase and chymase [2]. These unique mast cell enzymes constitute ~ 30% of the total granule protein and exhibit substrate specificities that include protease activated receptors (PARs), tethered ligand receptors that are linked to epithelial, endothelial and fibroblast activation and/or proliferation [2,3].

The mast cell in the airway submucosa is positioned at the interface between the external and internal environments to

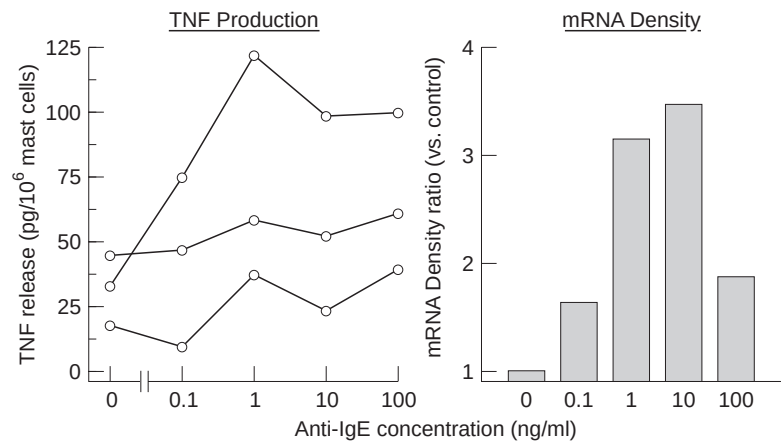
enable it to respond rapidly to stimuli with mediator and cytokine secretion. In also being a cell with prolonged survival, the mast cell is also able to respond to the local microenvironment with chronic mediator release. For example, human mast cells respond to stem cell factor (SCF, c-kit ligand) that is secreted by fibroblasts and epithelial cells which enhances their survival, primes them for IgE-dependent mediator release and, at higher concentration, initiates mediator release itself in the absence of IgE [4]. Tumour necrosis factor (TNF α), one of the pleiotropic cytokines secreted by activated mast cells [1,5] (Fig. 1) not only enhances the adhesion molecule cascade responsible for neutrophil and eosinophil recruitment during the late asthmatic response to allergen [6] (Table 2), but, by stimulating TNF α R₁ on the surface of mast cells in an autocrine manner further enhances mediator release by a mechanisms involving the activation of the transcription factor nuclear factor κ -B (NF- κ B) [7].

The primed mast cell will also respond to physical stimuli, especially a change in osmolality [8]. In exercise-induced asthma, there is evidence that mast cells become activated for mediator secretion as reflected by an increased excretion of urinary metabolites of prostaglandin D₂ and cysteinyl leukotrienes [9]. The features of hyperosmolar mast cell

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Table 1. Mediators secreted by mast cells

Granule mediators	Histamine, heparin, tryptase, chymase, carboxypeptidase A, stromolysin 1 (MMP-3)
Granule and newly formed cytokines	IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, L-13, TNF α
Granules and newly formed growth factors	GM-CSF, b-FGF, SCF
Granule and newly formed chemokines	IL-8, RANTES, MCP-1, MCP-3, MCP-4, eotaxin
Newly formed arachidonic acid mediators	PGD ₂ , LTC ₄
Other mediators	Adenosine, O ₂

Fig. 1. The dose-dependent release of TNF α (left) and mRNA transcription of TNF α (right) in highly purified human lung mast cells activated by anti-IgE.

activation are very similar to those induced by adenosine via the A_{2b} receptor [10]. Like exercise or hyperosmolar challenge, inhaled adenosine (or its 5'-monophosphate) causes an acute bronchoconstrictor response linked to the release of histamine, prostanoids and cysteinyl leukotrienes. Cross refractoriness exists between exercise and adenosine suggesting that they might share the same intracellular pathways to initiate or enhance mast cell mediator secretion.

Based on the sentinel role that the mast cell plays in asthma, it is a logical target to direct pharmacotherapy. The first drug to be introduced that was considered to exert its actions via mast cell inhibition was sodium cromoglycate.

Table 2. Effects of TNF α

Lymphocytes
Increased MHC expression
Increased antigen presentation
NK cell activation
Endothelium
Increased adhesion protein expression
Eosinophils
Increased activation
Cytokines
Synergy with IL-1 and IFN-gamma

Roger Altounyan showed this derivative of the herbal extract keillin to block both the early and late phase of bronchoconstriction with allergen [11]. Subsequent work by Cox and colleagues showed that sodium cromoglycate could also inhibit mediator secretion from activated rat mast cells [12].

Sodium cromoglycate and asthma

The discovery of this unique 'mast cell stabilizing' drug led to the first clinical trial of its regular inhalation as a treatment for asthma, an indication reinforced by its known inhibitory actions on allergen-induced responses in asthma [11]. In 1967 Howell and Altounyan published the first 6-week clinical trial of this drug on inhaled sodium cromoglycate administered by Spinhaler[®] and demonstrated clinical benefit [13]. This was the first inhaled 'controller' drug introduced to treat asthma and only later followed by inhaled corticosteroids.

Following the introduction of this cromone into clinical practice, studies conducted over the 20 years showed that it inhibited allergen, exercise, hypertonic, irritant, neuropeptide, adenosine and hypo-osmolar airway responses in asthma [14]. Similar results were obtained with a second compound, nedocromil sodium, which was slightly more efficacious on some of the challenges [15].

In rodent mast cells phosphorylation of a 78-kDa protein was shown to occur with both IgE-dependent mast cell activation and on exposing mast cells to cromones and led to the suggestion that this intracellular protein was linked to a natural inhibitor of mast cell function. Recently the 78 kDa protein has been identified as moesin, a protein of the ezrin-radixin group involved in the intracellular coupling of IgE-receptor signalling to the cytoskeletal network necessary to initiate degranulation [16]. Since CD44 ligation also activates this network in epithelial cells [17], it has been suggested that CD44 itself is the 'receptor' for cromones.

Despite many clinical trials of these two drugs both in children and in adults, their widespread use as controller agents has been difficult because of large differences in efficacy. This has led to the concept of the 'responder' and 'non-responder' cromones. Only 20–30% of adult asthmatic patients and rather more children appear to respond well to drugs in this class. Thus, within the spectrum of asthma, there are important differences in therapeutic responses to pharmacological agents reflecting disease heterogeneity with mechanistic significance. Presumably, those who respond best to cromones have a large mast cell component. This view is strengthened by an association between efficacy and a strong allergic component to the asthma as found in children and young adults [18].

Another possibility that helps to explain the suboptimal efficacy of cromones in asthma is that the dose response relationship is shallow and variable. When studied on human mast cells *in vitro*, sodium cromoglycate or nedocromil sodium rarely produced total inhibition of mast cell mediator release and *in vitro* are substantially less active than β_2 -adrenoceptor agonists at inhibiting mast cell mediator release [19]. Thus, in recent asthma guidelines, it has been argued that the relatively low overall efficacy of cromones on mast cell function precludes their widespread use as controller anti-asthma treatments. In their favour, however, is the remarkably safe profile of these drugs.

Targets for future mast cell stabilizing drugs

The low overall efficacy of cromones in treating asthma on a population basis would be improved if drugs could be found that could totally inhibit mast cell mediator release. The recent discovery of proteins that can effectively interfere with mast cell IgE receptor signalling has created this opportunity [20]. Thus, the identification of mast cell associated function antigen (MAFA), gb49b1 and Fc ϵ RIIB receptors which contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) creates new molecular targets for mast cell inhibition [21]. These proteins associate with the Fc ϵ R1 complex in membrane rafts to potentially inhibit mast cell activation by recruiting sH-1 and sH-2 which

dephosphorylate the β and γ chains of the Fc ϵ R1 ($\alpha\beta\gamma_2$) [20].

The importance of inhibiting mast cells as a target for asthma has recently received a strong boost following studies with the non-anaphylactogenic humanized monoclonal antibody E-25 which is directed to an epitope on the C₃ domain of the F_c portion of IgE [22] and blocks its binding to the α -chain of Fc ϵ R1 and to the low affinity receptor (Fc ϵ R₂, CD23). When administered to asthmatic patients over 9 weeks, this anti-IgE monoclonal antibody almost completely inhibited the early and late phase to inhaled allergen and bronchoconstrictor responses also reduced the acquired increase in bronchial hyperresponsiveness and influx of eosinophils into the airways [23,24]. Of considerable interest is that this treatment also reduced the level of expression of Fc ϵ R1 on basophils and, by inference, mast cells also [25]. A 12-week clinical trial in patients with moderate–severe asthma some of whom were receiving inhaled corticosteroids and some oral corticosteroids, revealed a marked improvement in both pulmonary function and clinical indices of asthma [22]. Of considerable interest is the observation that all patients taking regular oral corticosteroids were able to tail off this treatment and those taking high-moderate doses of inhaled corticosteroid were able to reduce this therapy by 50%.

Although it is possible that E-25 produces its efficacy via cells other than mast cells (and basophils), this does not seem likely since the treatment had profound effects on early and late responses to allergen challenge which are known to be mast cell dependent. The efficacy of E-25 was dependent upon reducing the circulating IgE concentration by >98% and required regular (weekly) injections. However, its clear effectiveness strengthens the view that targeting mast cells (and basophils) is justifiable for a drug discovery strategy in asthma and related disorders.

However, as more and more selective drugs are being developed to target specific cells and bronchial pathways in asthma, the reality of responders and non-responders will need to be faced. As with the cromones, it is likely that many of the new drugs will be active in only a proportion of patients but, with improved drug potency and efficacy, hopefully a greater proportion.

Discussion following Sampson and Holgate

Barnes: In a recent study we performed, following one week of treatment with placebo, beclomethasone dipropionate (BDP) 400 μ g/day or zafirlukast, patients underwent an allergen challenge which produced an early and a late response, and 24 h later had a bronchial biopsy and bronchoalveolar lavage (BAL). In the placebo group, there was no change in the number of neutrophils in the biopsy, but in the BDP-treated group there was a statistically significant rise in

the number of neutrophils in the biopsy. This agrees with what Tony Sampson was saying, that in the severe asthmatics it may not be the disease but the drug that is causing the neutrophilia. Peter Jeffery has been involved in a study, which had similar findings. So there are now at least two studies that show that it may be the steroids rather than the disease that is causing the neutrophilia.

Holgate: The critical question is whether those neutrophils are doing anything?

Redington: Can I just make a comment on this neutrophil issue. My understanding of the antileukotrienes is that the clinical effectiveness of the leukotriene receptor antagonists (which will only antagonize LTC₄, LTD₄ and LTE₄) and of the synthesis inhibitors (which will also inhibit LTB₄) are very similar. Presumably that could be argued as evidence against a role for neutrophils.

Sampson: There has not been a single head-to-head comparison of the leukotriene receptor antagonists and the leukotriene synthesis inhibitors, so we just don't know. Also, zileuton, the only extensively tested synthesis inhibitor, is not a terribly powerful drug. In nocturnal asthma, Sally Wenzel [26] found that zileuton effectively decreased elevated LTB₄ from BAL fluid and reduced neutrophils in BAL fluid. The reduction in neutrophils correlated well with the clinical effects of zileuton.

Bousquet: I had some difficulty in following completely your presentation but I think you said that apoptosis was induced in neutrophils by corticosteroids.

Holgate: No, it is blocked.

Bousquet: Does this not contradict the papers of Chris Haslett [27] who studied neutrophils and eosinophils, and found a significant difference between the two.

Sampson: That is the point. Steroids promote apoptosis in eosinophils but delay it in neutrophils. It is one of the mechanisms for blood neutrophilia.

Szczeklik: How is mast cell tryptase inhibited in tissues?

Holgate: It is not known. It seems to be regulated by its ability to stay as a tetramer. Once the tetrameric enzyme disintegrates, particularly if calcium is present or the heparin molecule is removed, it dramatically unfolds and the active site becomes concealed. But with the recombinant human enzyme one can generate monomers that are active. What is not known is whether the enzyme can exist in the tissue *in vivo* as an active monomer. No endogenous inhibitors of tryptase have yet been described. It is a very strange enzyme that is shaped like a doughnut and it will only cleave very selective substrates as a consequence of this. It is very different from trypsin in that regard. This whole protease activated receptor (PAR) system is going to become quite an interesting field generally in relation to cell signalling triggered by proteolytic enzymes. We are going to find different PARs are activated and cleaved by different enzymes. It appears

that PAR2 is the one that is picked off selectively by tryptase.

Joos: I have a question about interaction with smooth muscle because in your last scheme the mast cell very close to smooth muscle.

Holgate: Tunon de Lara in Bordeaux has generated some really interesting data showing that tryptase can actually stimulate smooth muscle cells. This is similar in a way to thrombin, which is one of the standard reagents for proliferation. There may be a variety of PAR receptors in smooth muscle that may be activated and that changes the function of the smooth muscle.

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