Mast cell activation

Monitoring mast cell activation by prostaglandin D_2 in vivo

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Prostaglandin D_2 is a useful in vivo marker of mast cell activation in humans

While the pro-inflammatory role of eosinophilic granulocytes in asthma is currently under debate, an increasing body of evidence suggests that mast cells may indeed orchestrate many of the characteristic pathophysiological changes in asthma.[1] There are also indications that the mast cell may be an effector cell in other lung diseases such as chronic obstructive pulmonary disease[2–4] and lung fibrosis.[5] Given the location of mast cells at multiple sites within the airways,[1] they clearly have the potential to function as sensors of alterations in the microenvironment—be it to inhaled or bloodborne substances, microbes, or other insults that require a prompt host defence reaction. Their versatility is demonstrated by the great number of stimuli that trigger mast cell activation (fig 1). In addition to classical IgE dependent degranulation of mast cells, transduction pathways resulting in mast cell activation may be triggered by, for example, adenosine,[6] hyperosmolarity,[7] and lipopolysaccharide.[8]

MAST CELL MARKERS

Although many mast cell mediators or products serve as useful markers of mast cell activation in vitro, it has been notoriously difficult conclusively to establish mast cell activation in human studies. For example, it is difficult to catch the short lived increase in plasma levels of histamine and its metabolites following allergen induced bronchoconstriction. Furthermore, circulating basophils may contribute significantly to plasma histamine[9] and plasma values may be increased by non-specific challenges such as an ordinary exercise test. Measurements of urinary metabolites of histamine may sometimes be helpful to provide information regarding systemically released histamine over time[10] but, due to extensive metabolism, only a small percentage of circulating histamine levels appear in the urine and the ambiguity with regard to the cellular source remains.

Tryptases, which are proteases secreted by degranulating human mast cells, have been reported to make up about 25% of total mast cell protein.[11] This would seem to make tryptase an ideal marker of mast cell activation. Although tryptase measurements are very useful in experimental work with cells and tissues, this marker has not been particularly helpful in mechanistic studies addressing mast cell activation in humans. This may relate to limitations in the currently available methodology for measuring plasma or serum tryptase. Nevertheless, so far, the main uses of tryptase measurements are to provide evidence for the diagnosis of systemic mastocytosis or necropsy evidence of systemic anaphylaxis.[12]

Prostaglandins (PG) are ubiquitously biosynthesised and would therefore seem to be unlikely candidates as specific markers for any particular cell. However, in this issue of *Thorax*, Bochenek *et al*[13] confirm and extend the accumulated evidence that measurement of PGD_2 or its metabolites represents a sensitive and reliable strategy for assessment of mast cell activation in vivo. Specifically, they convincingly show, for the first time, increased levels of the primary PGD_2 metabolite 9α11β-PGF_2 in plasma during the early phase of allergen induced airway obstruction. This is achieved by applying gas chromatography-negative ion chemical ionisation-mass spectrometry (GC-NICI-MS) to samples collected at frequent intervals before and during allergen bronchoprovocation of subjects with atopic asthma. The methodology is very appropriate as GC-NICI-MS is the most specific measurement of this particular family of compounds, where the presence of numerous structurally related metabolites always complicates immunoassay measurements. Bochenek *et al* also deserve credit for their development of a protocol that improves the sensitivity of the GC-NICI-MS measurements.

BIOSYNTHESIS OF PGD_2 IN MAST CELLS

The release of PGD_2 from isolated human mast cells was reported more than two decades ago,[14] shortly followed by the demonstration of its release into human airways after local endotracheal instillation of allergen.[15] However, the mechanistic significance of these reports was not generally appreciated. In humans, mast cells are an almost exclusive cellular source of PGD_2.[16] Although there is evidence of some PGD_2 formation by platelets, macrophages and certain T lymphocytes,[13] the reported amounts are 100–1000 times lower than those produced during IgE dependent activation of mast cells. More importantly, whereas the basophil and the mast cell both release histamine and leukotriene (LT) C_4, it is only the mast cell that produces significant quantities of PGD_2.[16] There is, in fact, recent evidence to show that increased expression of the haematopoetic PGD_2 synthase may be the functional response that is most specifically upregulated in activated mast cells.[17]

MEASUREMENT OF PGD_2

The currently renewed interest in applications of PGD_2 measurement would not have been possible without the comprehensive work of Roberts and colleagues at Vanderbilt who performed painstaking GC/MS identifications of PGD_2 metabolites in blood and urine after injections of radiolabelled PGD_2.[18 19] More than 25 metabolites were identified but intact PGD_2 was not found in the urine. The most abundant PGD_2 metabolite identified was 9,11-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioc acid, commonly referred to as PGD-M. The earliest appearing urinary metabolite was 9α,11β-PGF_2, which was subsequently shown to be stereospecifically transformed from PGD_2 by the NADPH dependent enzyme 11-ketoreductase[20] in lung and liver. Interestingly, 9α,11β-PGF_2 retains biological activity. It has, for example, been found to contract bronchial smooth muscle[21] and has vascular effects including contraction of coronary arteries.[22] Metabolism of 9α,11β-PGF_2 by the 15-hydroxy prostaglandin dehydrogenase, followed by β- and ω- oxidations, leads to PGD-M.

The Vanderbilt group thus used GC/MS measurements of PGD-M as a marker of systemic PGD_2 production in different disease states. Markedly raised levels of PGD-M were discovered in systemic mastocytosis[23] as well as during anaphylaxis. The GC/MS approach is, however, laborious and technologically demanding, which generally renders it less applicable to studies of populations and large numbers of samples. The more recent validation of an immunoassay method for the measurement of 9α,11β-PGF_2 in urine[24 25] has therefore created new opportunities for

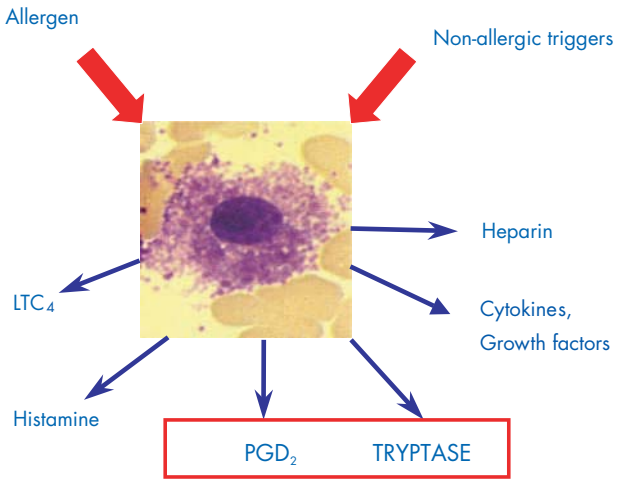

Figure 1 Mast cells may produce a large number of mediators, enzymes, cytokines and other factors in response to allergic (IgE dependent) or non-allergic activation (adenosine, exercise, endotoxin, mannitol, non-steroidal anti-inflammatory drugs (NSAIDs) in NSAID intolerant subjects, etc). However, only tryptase and prostaglandin (PG) D_2 (boxed) are specific markers of mast cell activation. As reported by Bochenek *et al* in this issue, measurement of PGD_2 and its metabolites is currently the most sensitive strategy to monitor mast cell activation in human subjects. LTC_4 = leukotriene C_4.

using this PGD_2 metabolite as a mast cell marker. Using this immunoassay methodology, increased excretion of metabolites of PGD_2 into the urine has been observed after allergen induced bronchoconstriction[10 24] and mast cell involvement in other indirect challenges has also been confirmed.[24 26 27]

As discussed by Bochenek *et al*,[13] apart from weak indirect or anecdotal evidence, there has not previously been any investigation of 9α,11β-PGF_2 levels in plasma during allergen induced bronchoconstriction, which undoubtedly must be the gold standard for mast cell activation. Interestingly, the current demonstration of increased PGD_2 release during allergen induced bronchoconstriction puts further weight behind a previous publication from the group in Krakow where increased plasma levels of 9α,11β-PGF_2 were found following aspirin induced bronchoconstriction.[28] This adds to several other lines of evidence[24] suggesting that the intolerance to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) involves mast cell activation.

Bochenek *et al* confirmed the original observations by Liston *et al*[18] that 9α,11β-PGF_2 was the first PGD_2 metabolite to appear in urine, although they did not find the increase of this metabolite in the urine to be as great as that reported by O'Sullivan *et al*.[10] These seemingly different findings are most probably explained by the demonstration[25] that the immunoassay measures not only 9α,11β-PGF_2 but also at least two other metabolites that appear somewhat later. In other words, for detection of the sum of the initially excreted PGD_2 metabolites in urine, the chemically less specific immunoassay will paradoxically have greater practical sensitivity as it measures several related PGD_2 metabolites. However, as pointed out by Bochenek *et al*, for studies of the kinetics of 9α,11β-PGF_2 metabolism, the chemically more specific method is obviously preferable.

PERSPECTIVES

The method chosen to monitor mast cell activation by measurement of PGD_2 metabolites will obviously depend on the questions asked and the resources available. Irrespective of the analytical method selected, measurements of 9α,11β-PGF_2 in plasma, urine, or other body fluids currently provide the most sensitive method for detection of mast cell activation in vivo. This was clearly shown in the paper by Bochenek *et al*, where there was no change in plasma tryptase despite the fivefold increase in plasma 9α,11β-PGF_2. Similarly, in previous work by O'Sullivan *et al*,[10 26] there was consistently a much smaller or non-significant increase in urinary methyl histamine in contrast to consistent and prominent increases in urinary 9α11β-PGF_2 metabolites. Thus, for investigations into the role of the mast cell in different pulmonary diseases, measurements of PGD_2 metabolites in body fluids offer many new opportunities.

Finally, PGD_2 is not only a marker of mast cell activation but also—together with its immediate metabolite 9α,11β-PGF_2—it is a potent mediator of bronchoconstriction, vasomotor tone, and cell recruitment.[29] We hypothesise that PGD_2 mediates the component of allergen induced bronchoconstriction that remains resistant to antihistamines and antileukotrienes.[30] Experimental data are available to support such a role,[29 31] and a role for PGD_2 in rhinitic responses in humans has also been implicated.[32] The recent awareness that there are at least three different receptors (TP, DP, and CRTH2) mediating the effects of PGD_2 in the airways[29] suggests that we may soon get improved opportunities to define more precisely the pulmonary role of this mast cell derived mediator.

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Breathlessness during exercise in COPD

Breathlessness during exercise in COPD: how do the drugs work?

P M A Calverley

Salmeterol reduces breathlessness during exercise without necessarily changing exercise duration

The inability to exercise because of distressing breathlessness is one of the most frequent problems experienced by patients with chronic obstructive pulmonary disease (COPD)[1] and is a major determinant of impaired quality of life.[2] Our understanding of why this occurs and how best to treat it has improved significantly in the last decade. At one level the problem appears relatively straightforward. Exercise invariably involves an increase in whole body oxygen consumption and carbon dioxide production, which requires an appropriate rise in alveolar ventilation if arterial carbon dioxide tension is to remain constant. In patients with COPD the ability to increase minute ventilation is restricted as is the capacity to empty their lungs quickly, hence exercise limitation occurs at a lower workload than in age matched healthy subjects. Although there is much truth in this simple scheme, it does not do justice to the many complex adaptive responses that patients use to cope with their chronic airflow obstruction, nor does it explain the variability seen in both the duration of exercise and the intensity of breathlessness in patients with apparently similar levels of airflow obstruction.

ADAPTATIONS TO REDUCED EXERCISE CAPACITY IN COPD

Unsurprisingly, the general relationship of ventilatory capacity, commonly established indirectly from the forced expiratory volume in 1 second (FEV_1),[3] is not simple. While the 12 minute walking distance is broadly related to the severity of airflow obstruction,[4] spirometric measurements are not very precise indicators of exercise capacity in the individual patient. Differences in ventilation-perfusion matching during exercise mean that individuals with more ''wasted ventilation'' achieve their maximum sustainable ventilation sooner for the same degree of alveolar ventilation.[5] Differences in pre-morbid fitness are also relevant. Patients who are less fit progress to anaerobic metabolism (and hence increased carbon dioxide production) at lower levels of work than those who are fitter.[6] More recently, differences in the dynamic behaviour of the respiratory system during exercise have been identified.[7] Unlike healthy subjects, patients with severe COPD progressively increase their end expiratory lung volume rather than reducing it during exercise as occurs in healthy subjects. Since total lung capacity is constant, these patients have a restricted ability to increase tidal volume and their minute ventilation is increased predominantly by an increase in respiratory frequency. The ability to eliminate carbon dioxide is even worse in these circumstances and some patients become hypercapnic before stopping exercise.[8] More recently, changes in end expiratory chest wall volume—which reflects changes in lung volume—have been shown to occur during uninstrumented exercise.[9] This pattern of response is not universal as some patients appear to retain the more normal behaviour of trying to reduce end expiratory lung volume while exercising, which perversely may be a bad strategy when they are close to flow limitation at rest. Nonetheless, patients with the most severe COPD, and certainly those with the lower expiratory flow reserve, adopt a strategy of allowing dynamic hyperinflation to occur which further compromises their respiratory mechanics and increases their sensation of breathlessness.

Changes in lung volume with exercise provide an attractive explanation for the