Mast cells, cytokines and asthma

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The appreciation that asthma is a chronic inflammatory disorder of the airways has led to a reappraisal of the importance of different cell populations within the bronchial mucosa with respect to their role in the regulation of the cellular events in this disease. While mast cell degranulation has been implicated in the acute allergic bronchoconstrictor response, activation of this cell population has not been considered relevant to either the late phase inflammatory cell influx within the airways following allergen bronchoprovocation or to the mucosa eosinophilia in chronic clinical disease. As such, attention has focused on the T lymphocyte as an orchestrator of these cellular events on account of its ability to synthesize and release cytokines relevant to the allergic process. It is now, however, realized that many cell populations within the airways are able to generate cytokines comparable with and complimentary to those produced by T lymphocytes and that asthma cannot be considered an inflammatory airway disorder dependent upon activation of one single cell population. This review details the current evidence that airway mast cells synthesize, store and release cytokines relevant to allergic inflammation and considers their potential involvement not only in the cellular influx within the airways but also in the fibrotic structural changes which are evident in chronic disease.

Key Words: Asthma, Inflammation, Interleukin-4, Interleukin-5, Interleukin-6, Mast cells, Tumour necrosis factor-alpha

Les mastocytes, les cytokines et l’asthme

RÉSUMÉ : L’appréciation de l’asthme comme trouble inflammatoire chronique des voies aériennes a entraîné une réévaluation de l’importance des diverses populations de cellules dans la muqueuse bronchique quant à leur rôle régulateur des événements cellulaires se produisant dans cette maladie. Si la dégranulation des mastocytes a été impliquée dans la réaction bronchospastique allergique immédiate, le rôle de l’activation de cette population de cellules dans l’afflux cellulaire inflammatoire de la réaction retardée dans les voies aériennes suite à un test de provocation bronchique avec un allergène, et dans l’éosinophilie de la muqueuse identifiée dans la maladie chronique, n’a pas été retenu. À ce titre, l’attention s’est portée vers le lymphocyte T comme cellule orchestre de ces événements, à cause de sa capacité à synthétiser et à libérer des cytokines intervenant dans le processus allergique. Cependant, on sait maintenant que de nombreuses populations de cellules dans les voies aériennes peuvent générer des cytokines comparables et complémentaires à celles produites par les lymphocytes T. Il s’ensuit que l’asthme ne peut être considéré comme un trouble inflammatoire des voies aériennes tributaire de l’activation d’une seule population de cellules. Cette revue présente en détail les données actuelles démontrant que les mastocytes des voies aériennes synthétisent, emmagasinent et libèrent des cytokines jouant un rôle dans l’inflammation allergique et considère qu’ils participent probablement à l’afflux cellulaire dans les voies aériennes, mais aussi à la constitution de lésions de fibrose observées dans la maladie chronique.
HISTORICALLY, THE MAST CELL HAS HAD A LONG ASSOCIATION WITH ASTHMA, DATING FROM THE EARLY IDENTIFICATION OF HISTAMINE AS A MEDIATOR OF ANAPHYLACTIC REACTIONS (1) AND ITS SUBSEQUENT LOCALIZATION TO THE MAST CELL (2). MAST CELLS ARE FOUND THROUGHOUT THE RESPIRATORY TRACT WHERE THEY ARE PRESUMED TO PLAY A PHYSIOLOGICAL ROLE IN HOST DEFENCE. THEY ARE LARGE CELLS, 5 TO 15 µM IN DIAMETER, AND CONTAIN WITHIN THEIR CYTOPLASM NUMEROUS SECRETORY GRANULES WHICH HAVE CHARACTERISTIC SCROLL, LATTICE AND GRATING APPEARANCES WHEN VIEWED BY TRANSMISSION ELECTRON MICROSCOPY. THESE GRANULES CONTAIN HISTAMINE AND NEUTRAL PROTEASES SUCH AS TRYP TASE AND CHYMASE, ALL OF WHICH ARE STORED IN A PREFORMED STATE AND WHICH ARE RAPIDLY RELEASED IN RESPONSE TO CROSS-LINKAGE OF CELL SURFACE-BOUND IMMUNOGLOBULIN (Ig) E BY ALLERGEN. IN ADDITION, MAST CELL ACTIVATION LEADS TO THE RELEASE OF NEWLY GENERATED LIPID MEDIATORS, INCLUDING PROSTAGLANDIN (PG)D2 AND THE SULFIDOP EPTIDE LEUKOTRIENES, LEUKOTRIENE (LT)C4 AND ITS METABOLITES LTDA4 AND LTE4. IN COMPARISON WITH HISTAMINE, LTC4, LTD4 AND LTE4 ARE EXTREMELY POTENT SPASMOMGENIC MEDIATORS, AND THEY CONSTITUTE THE ACTIVITY PREVIOUSLY KNOWN AS ‘SLOW REACTING SUBSTANCE OF ANAPHYLAXIS’ (3). THERE IS GOOD EVIDENCE THAT THESE SPASMOMGENIC MEDIATORS ALONG WITH PGD2 ARE RESPONSIBLE FOR THE EARLY BRONCHOCONSTRICTOR RESPONSE SEEN IN SENSITIZED ASTHMATICS FOLLOWING ALLERGEN CHALLENGE. THESE MEDiators ARE RECOVERED IN INCREASED QUANTITIES FOLLOWING LOCAL ENDOBRONCHIAL ALLERGEN CHALLENGE IN ASTHMA (4-6), AND HISTAMINE (7), PGD2 (8) AND LEUKOTRIENE RECEPTOR ANTAGONISTS (9) ALL ATTENUATE THE AIRWAY RESPONSE TO ALLERGEN.

DURING THE PAST DECADE THE CONCEPT OF ASTHMA AS A CHRONIC INFLAMMATORY DISORDER HAS BECOME WIDELY ACCEPTED. EARLY EVIDENCE IN SUPPORT OF THIS CAME FROM A NUMBER OF STUDIES EXAMINING EITHER POST-MORTEM TISSUE FROM PATIENTS WHO HAD DIED OF ACUTE SEVERE ASTHMA (10-13) OR BIOPSIES OBTAINED FROM LIVING ASTHMATICS USING RIGID BRONCHOSCOPY (14,15). MORE RECENTLY THE AVAILABILITY OF FIBREOPTIC BRONCHOSCOPY AS A MEANS TO OBTAIN STEMENS AND THE DEVELOPMENT OF MORE RIGOROUS METHODS OF QUANTIFICATION HAVE ENABLED A DETAILED CHARACTERIZATION OF THE PATHOLOGICAL FINDINGS NOT ONLY IN ALLERGIC ASTHMA (16-21) BUT ALSO IN OTHER FORMS SUCH AS INTRINSIC (22) AND OCCUPATIONAL (23,24) ASTHMA. TAKEN TOGETHER THESE STUDIES HAVE REVEALED THAT, IRRESPECTIVE OF THE PRESUMED ETIOLOGY, A KEY PATHOLOGICAL FEATURE OF ASTHMA IS THE PRESENCE OF A CHRONIC INFLAMMATORY INFILTRATE WITHIN THE AIRWAY.

THIS CHANGE IN EMPHASIS FROM VIEWING ASTHMA AS AN ABNORMALITY OF BRONCHIAL SMOOTH MUSCLE TONE TO A DISORDER CHARACTERIZED BY CHRONIC INFLAMMATION HAS BEEN ACCOMPANIED BY A DECLINE IN THE IMPORTANCE ATTACHED TO THE IMMEDIATE AIRWAY RESPONSE TO ALLERGEN CHALLENGE AND BY INERENCE TO THE MAST CELL. AS SHORT ACTING BETA-AGONISTS, WHICH IN VITRO INHIBIT IgE-DEPENDENT ACTIVATION OF HUMAN LUNG MAST CELLS (25), HAVE NO INFLUENCE ON THE EARLY AIRWAY RESPONSE FOLLOWING ALLERGEN CHALLENGE (26), IT HAS BEEN CONSIDERED THAT THE MAST CELL DOES NOT PLAY A SIGNIFICANT PART IN EVENTS LEADING TO THE DEVELOPMENT OF AIRWAY INFLAMMATION AND BRONCHIAL HYPERREACTIVITY. SIMILARLY, AS SHORT ACTING BETA-AGONISTS DO NOT IMPROVE THE LONG-TERM PATTERN OF ASTHMA SEVERITY (27), AND AS NOVEL ‘ANTI-ALLERGIC’ COMPOUNDS, WHICH INHIBIT MAST CELL DEGRANULATION IN ANIMAL MODELS, HAVE FAILED TO DEMONSTRATE CLINICAL EFFICACY IN HUMAN ASTHMA (28), IT HAS ALSO BEEN CONSIDERED THAT MAST CELL ACTIVATION DOES NOT CONTRIBUTE SIGNIFICANTLY TO THE PATHOGENESIS OF AIRWAY INFLAMMATION IN CLINICAL ASTHMA. THERE IS GOOD EVIDENCE THAT THESE SPASMOMGENIC MEDIATORS ALONG WITH PGD2 ARE RESPONSIBLE FOR THE EARLY BRONCHOCONSTRICTOR RESPONSE SEEN IN SENSITIZED ASTHMATICS FOLLOWING ALLERGEN CHALLENGE. THESE MEDiators ARE RECOVERED IN INCREASED QUANTITIES FOLLOWING LOCAL ENDOBRONCHIAL ALLERGEN CHALLENGE IN ASTHMA (4-6), AND HISTAMINE (7), PGD2 (8) AND LEUKOTRIENE RECEPTOR ANTAGONISTS (9) ALL ATTENUATE THE AIRWAY RESPONSE TO ALLERGEN.

However, while T lymphocyte activation and the potential for cytokine production by these cells are increased in asthma, it is now appreciated that the selective focus on one particular cell is misplaced as many cell types have the potential to contribute to the ‘cytokine pool’ within the airways. Mast cell activation is known to occur in clinical asthma, with ultrastructural features on transmission electron microscopy of partial degranulation (16, 29) (Figure 1), evidence of increased histamine release by bronchoalveolar lavage (BAL) fluid mast cells from asthmatics ex vivo (30, 31) and the identification of increased recovery of mast cell mediators in BAL fluid (5, 32, 33). The recognition that, in addition to these ‘classical’ mediators, mast cell activation can also lead to the release of cytokines has renewed interest in the potential involvement of this cell type in the orchestration of airway inflammation.

CYTOKINE PRODUCTION BY MAST CELLS

The initial investigations of cytokine generation by mast cells were performed using long term murine mast cell lines. Abelson murine leukemia virus (AbMuLV)-transformed mast cell lines were shown to produce the cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF) (34, 35), interleukin (IL)-3 (35) and IL-4 (36). Subsequently it was reported that nontransformed IL-3-dependent mast cell lines were able to generate IL-3, IL-4, IL-5 and IL-6 when stimulated by cross-linkage of FceR1 (37). These observations were confirmed, and the range of cytokines produced by activated murine mast cell lines was extended to include IL-1, IL-2, GM-CSF, interferon (IFN)γ and four members of mast cell. As short acting beta-agonists, which in vitro inhibit IgE-dependent activation of human lung mast cells (25), have no influence on the late airway response following allergen challenge (26), it has been considered that the mast cell does not play a significant part in events leading to the development of airway inflammation and bronchial hyperreactivity. Similarly, as short acting beta-agonists do not improve the long-term pattern of asthma severity (27), and as novel ‘anti-allergic’ compounds, which inhibit mast cell degranulation in animal models, have failed to demonstrate clinical efficacy in human asthma (28), it has also been considered that mast cell activation does not contribute significantly to the pathogenesis of airway inflammation in clinical asthma. Thus, recent emphasis has been directed towards the T lymphocyte, with its ability to synthesize cytokines, as the key cell in coordinating the cellular events that occur following allergen challenge and in clinical disease.

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TABLE 1
Cytokines known to be produced by human mast cells and their actions in relation to allergic inflammation

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Actions relevant to asthma</th>
</tr>
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<tbody>
<tr>
<td>TNF-α</td>
<td>Increases eosinophil cytotoxicity</td>
</tr>
<tr>
<td></td>
<td>Monocyte chemoattractant</td>
</tr>
<tr>
<td></td>
<td>Increases endothelial expression of ICAM-1, E-selectin and VCAM-1</td>
</tr>
<tr>
<td></td>
<td>Induction of bronchial hyperreactivity</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>Activates B lymphocyte by increasing expression of CD23 and class II major</td>
</tr>
<tr>
<td></td>
<td>histocompatibility complex</td>
</tr>
<tr>
<td></td>
<td>Induces isotype switching of B lymphocytes for immunoglobulin E synthesis</td>
</tr>
<tr>
<td></td>
<td>Increases endothelial expression of VCAM-1</td>
</tr>
<tr>
<td></td>
<td>Eosinophil chemoattractant</td>
</tr>
<tr>
<td>Interleukin-5</td>
<td>Enhances eosinophil differentiation</td>
</tr>
<tr>
<td></td>
<td>Prolongs eosinophil survival</td>
</tr>
<tr>
<td></td>
<td>Eosinophil chemoattractant</td>
</tr>
<tr>
<td></td>
<td>Primes eosinophils for increased functional activity</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Activation of T lymphocytes</td>
</tr>
<tr>
<td></td>
<td>Terminal differentiation of B lymphocytes</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>Chemoattractant for:</td>
</tr>
<tr>
<td></td>
<td>• neutrophils</td>
</tr>
<tr>
<td></td>
<td>• eosinophils (when primed by exposure to other cytokines)</td>
</tr>
<tr>
<td></td>
<td>• T lymphocytes</td>
</tr>
</tbody>
</table>

ICAM-1: Intercellular adhesion molecule-1; TNF-α: Tumour necrosis factor-α; VCAM-1: Vascular cell adhesion molecule-1

the chemokine family, monocyte chemoattractant protein-1/JE, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β and T cell activation antigen-3 (38).

Production of several of these cytokines was next demonstrated in primary cultures of murine bone marrow-derived mast cells and/or purified peritoneal mast cells in response to IgE-dependent activation, in particular IL-3 and GM-CSF (39), IL-1 and IL-6 (38) and tumour necrosis factor (TNF)-α (40). In these studies mRNA synthesis generally preceded cytokine release, which was not maximal until several hours after stimulation, and accordingly it was concluded that the cytokines were newly synthesized. In one study, however, it was shown that mast cell activation resulted not only in the rapid release of preformed TNF-α stored in granules, but also in an increase in TNF-α mRNA accompanied by further sustained release of newly synthesized TNF-α (41).

These findings have now in part been extended to human mast cells. Table 1 lists those cytokines currently recognized, together with some of their major actions which are believed relevant to allergic inflammation. TNF-α was the first cytokine to be clearly associated with human mast cells. This cytokine is able to increase the expression of endothelial adhesion molecules, which are believed to be important in the recruitment of inflammatory cells into the airways from the peripheral circulation, in particular E-selectin (42), intercellular adhesion molecule-1 (ICAM-1) (42,43) and, when acting in synergy with IL-4, vascular cell adhesion molecule-1 (VCAM-1) (44). In addition, it exerts a priming effect on eosinophils with respect to cytotoxic ability (45,46) and superoxide generation (46); it is chemotactic for monocytes (47); and it induces bronchial hyperreactivity in experimental animals (48,49). Purified human skin mast cells were first demonstrated to synthesize and release TNF-α in response to IgE-dependent activation (50). This cytokine is stored preformed in mast cell granules (51) and, using a skin culture system, the rapid release of TNF-α from these stores in response to anti-IgE or other stimuli was shown to lead to increased expression of E-selectin on the endothelium of adjacent postcapillary venules (51,52). Immunoreactive TNF-α has also been localized to human lung mast cells, using the techniques of both dual staining (53) and examination of adjacent sections (54) (Figure 2); the proportion of mast cells expressing this cytokine is increased in asthma suggesting a role in disease expression (54). It remains to be determined whether human mast cells can synthesize both soluble and membrane-associated forms of TNF-α, as has recently been reported for rodent mast cells (55).
A second cytokine produced by human mast cells is IL-4. Purified human lung mast cells contain preformed IL-4, which is released in response to IgE-dependent activation (56). Furthermore, immunoreactivity for this cytokine has been localized to mast cells in bronchial mucosal biopsies (56) and, as with TNF-α, there is some evidence that the proportion of mast cells expressing IL-4 is increased in asthmatics (54). IL-4 is likely to be important in the recruitment of inflammatory cells in allergic inflammation. It regulates leukocyte-endothelial adhesion by increasing the endothelial expression of VCAM-1, a molecule that may be of particular relevance to the recruitment of eosinophils through its interaction with the β1 integrin VLA-4 which is expressed on eosinophils but not neutrophils (44,57,58); IL-4 also acts as an eosinophil chemoattractant (59). In addition IL-4 activates B lymphocytes and is recognized to be a critical factor in isotype switching for IgE synthesis (60,61). This process has usually been considered to have an absolute requirement in vivo for the presence of T lymphocytes, which not only release the necessary soluble factors but also express essential cell surface signals, notably the CD40 ligand, which has now been cloned (62). However, the recent demonstration that human lung mast cells also express the CD40 ligand (63), taken in conjunction with their ability to release IL-4 and other costimulatory cytokines such as IL-6, raises the possibility that the direction of IgE synthesis by B lymphocytes in vivo may not be an exclusive property of T lymphocytes.

Immunoreactivity for interleukin-5 has also been localized to mast cells in bronchial mucosal biopsies from both asthmatics and normal subjects (54), and preliminary information indicates that in response to IgE-dependent stimulation purified human lung mast cells increase their expression of mRNA for IL-5 (64). IL-5 has several important biological properties in vitro in relation to eosinophils. This cytokine stimulates their differentiation (65-67); increases their survival (68,69) by means of delaying apoptosis (70); primes them for increased activity in a number of functional assays (68,71); and is also an eosinophil chemoattractant (72). In addition early reports suggested a role for IL-5 in IgE synthesis by B lymphocytes (73,74), although subsequent studies have not been able to confirm this.

Interleukin-6 is produced by the human mast cell line HMCl and IL-6 immunoreactivity has again been localized to mast cells in bronchial biopsies (54). This cytokine induces the terminal differentiation of B lymphocytes and their activation for Ig synthesis (74,75) and also stimulates the proliferation of T lymphocytes (76). In addition, it serves a costimulatory role in antigen presentation, and this action may be relevant in the context of the recent report that highly purified mast cells express class II major histocompatibility complex (MHC) and can apparently present antigens to T lymphocytes (77).

In addition to IL-4, IL-5, IL-6 and TNF-α, there are also reports of IL-8 in association with human mast cells. IL-8 was first described as a neutrophil priming agent and chemoattractant (78,79); it is now also recognized to be chemotactic for T lymphocytes (80) and, when they are primed by prior exposure to other cytokines such as IL-5, IL-3 and GM-CSF, for eosinophils (81,82). Stimulated HMCl cells have been shown to synthesize and release IL-8 (83). Immunoreactive IL-8 has also been localized to cytoplasmic granules in human skin mast cells following stimulation with anti-IgE, but no evidence was found of preformed IL-8 in unstimulated mast cells (83). IL-8 immunoreactivity in mucosal biopsies from asthmatics, on the other hand, is very largely confined to bronchial epithelial cells (84), which may reflect a lower degree of stimulation in chronic disease. Several forms of neutrophil chemoattractant activity have been described in the sera of patients with asthma. A high molecular weight form is detectable following allergen challenge, appearing within minutes and persisting for at least 24 h (85,86). It is believed to derive from mast cells because the time course of its appearance parallels that of histamine; in addition its release is inhibited by pretreatment with either sodium cromoglycate or salbutamol (86,87). Its identity is unknown, but the high molecular weight of 600 kDa has suggested that it represents a soluble factor conjugated to a serum protein, such as α2-macroglobulin, which is approximately this size and which can bind a number of cytokines. It is possible that IL-8 accounts for part of this activity, and the findings from murine mast cells allow speculation that other members of the chemokine family may also be involved.

**MAST CELL CYTOKINES AND ASTHMA**

The identification of cytokines relevant to allergic inflammation within mast cells raises the question of the relative contribution of this particular cellular source in relation to other potential sources of cytokine synthesis within the airways. Increased levels of TNF-α and IL-6 have been reported in BAL fluid from symptomatic compared to asymptomatic asthmatics (88) and, in a separate report, elevated BAL fluid levels of IL-4 were identified in allergic but not nonallergic asthma (89). The derivation of these cytokines has been investigated by in situ hybridization to examine cytokine mRNA expression in cells recovered by BAL. These studies identify increased numbers of cells expressing mRNA for TNF-α, IL-2, IL-4, IL-5 and GM-CSF, but not IFN-γ in asthma (90,91), and also an increase in the numbers of cells expressing IL-4, IL-5 and GM-CSF mRNA 24 h after allergen challenge (92). Dual fluorescence and immunomagnetic cell separation techniques have demonstrated that the BAL cells expressing IL-4 and IL-5 mRNA are predominantly CD2+ T lymphocytes. While such studies are informative about luminal events, they only provide an indirect reflection of events within the airway wall, however, because the cellular profile of BAL fluid and mucosal biopsies differs. In situ hybridization on bronchial biopsies identifies the presence of cells expressing IL-5 mRNA in those from symptomatic asthmatics but not those from asymptomatic asthma nor those from normal subjects (93). A significant increase in the number of cells expressing mRNA for IL-5 and GM-CSF, together with a trend towards increase in those expressing mRNA for IL-2 and IL-4, has also been demonstrated in bronchial biopsies obtained from atopic asthmatics 24 h after...
allergen challenge (22). Neither of these studies, however, determined the cellular provenance of these cytokines.

In the absence of firm information about the relative importance of the various potential sources of cytokines within the airway wall, it has frequently been assumed that IL-4 and IL-5 are derived mainly or exclusively from T lymphocytes. Evidence of the increased expression of these cytokines in asthma has been seen as supporting the concept that there is selective expansion of a subpopulation of T lymphocytes analogous to the Th2 subset described in long term murine T lymphocyte clones (94). TNF-α and IL-8, on the other hand, have both been considered to originate predominantly from the monocyte/macrophage population. One feature of cytokine biology that has become increasingly clear, however, is the apparent redundancy in the system, with a diversity of cell types capable of producing a particular cytokine. Thus, the addition of mast cells to the list of potential sources of cytokines within the airway wall raises the question of the significance of this in relation to allergic inflammation.

Although it would clearly be of interest to know whether cytokines of mast cell origin play an obligatory role in any of the pathophysiological manifestations of asthma, there is no direct evidence relating to this in humans. Moreover, the information from animal studies is conflicting. For example, W/W^v mice, which are genetically almost entirely deficient of mast cells, are unable to develop an IgE-dependent cutaneous late-phase response (in which the cellular infiltrate comprises neutrophils almost entirely) (95). On the other hand these mice are nevertheless able to mount an effective pulmonary eosinophilic inflammatory response to transnasally administered parasite extract despite their lack of mast cells (96). β2 adrenoreceptor antagonists, which are potent inhibitors of mast cell degranulation in vitro (25), do not prevent the development of a late response following allergen challenge in asthmatics (26), nor when taken regularly over several weeks do they reduce BAL fluid eosinophilia (97). However, the influence of these agents on cytokine release from human mast cells has not yet been investigated, and there is also evidence to suggest that tolerance to their effects on mast cells develops in asthma (98).

Their ability to store cytokines in a preformed state and to release them rapidly in response to IgE-dependent activation suggests that mast cells may be responsible for the initial cellular recruitment following allergen exposure. The immediate release of IL-4 and TNF-α, for example, would be expected to result in increased expression of adhesion molecules such as E-selectin, ICAM-1 and VCAM-1 in response to allergen challenge and consequent early recruitment of eosinophils and other leukocytes using these pathways. Consistent with this, in the cutaneous late-phase reaction an influx of inflammatory cells is evident as early as 2 h (99), which would seem too soon to be a result of the release of newly synthesized cytokines from T lymphocytes. In addition, continued cytokine release from mast cells in the presence of persistent antigenic stimulation, acting in conjunction with cytokines derived from other cellular sources such as T lymphocytes, may be important in maintaining the chronicity of the inflammatory response. Of particular relevance are the likely effects of mast cell cytokines on eosinophils. IL-4, IL-5 and IL-8 can all act as chemotactic stimuli, IL-5 prolongs their survival by delaying apoptosis, and both IL-5 and TNF-α increase their degree of functional activation.

Coincident with the chronic inflammatory response in asthma is a parallel process of healing and regeneration which may be accompanied by fibrosis. Best described is the deposition beneath the epithelium of a thick layer of connective tissue comprising primarily collagens types I, III and V and fibronectin (100). This is believed to be synthesized by a population of myofibroblasts which are normally present at this site but which are increased in number in asthma (101). Its pathophysiological significance is unclear, but it may serve as a marker for fibrogenic activity more generally within the airway, and moreover in small airways its mechanical effect will be proportionately greater so that it may itself adversely influence airway function.

 Mast cell mediator release may be involved in this fibrotic response. Mast cells have been implicated in several other diseases involving fibrosis, including scleroderma (102) and certain forms of pulmonary fibrosis (103). The classical mast cell degranulation products, histamine (104), heparin (105) and tryptase (106), all have the potential to contribute while cytokine production by mast cells may also be implicated. In particular, TNF-α has been shown to be mitogenic for fibroblasts both in vitro and in vivo (107), and IL-4 stimulates fibroblasts both to proliferate (108) and to synthesize matrix proteins such as types I and III collagen and fibronectin (109). However, the most potent profibrotic cytokine known is transforming growth factor-β. Transforming growth factor-β mRNA has been described in murine mast cells (110), and canine mastocytoma cells have been reported to release TGFβ1 (111), but these findings have not yet been extended to human mast cells.

**ANTI-INFLAMMATORY THERAPY AND MAST CELL CYTOKINE PRODUCTION**

Corticosteroids exert a potent anti-inflammatory effect in asthma and form a mainstay of therapy. Several studies have shown that, in addition to reducing symptoms and improving physiological indices of lung function, they also decrease mucosal inflammation. Following a six-week course of beclomethasone dipropionate, for example, there was decreased T lymphocyte activation in BAL fluid (112) and reduced numbers of T lymphocytes, eosinophils and mast cells in bronchial biopsies (113). BAL fluid levels of the mast cell products histamine and tryptase were also reduced (114) but, as histamine release from purified mast cells is not affected by dexamethasone (115), this may be secondary to reduced mast cell numbers rather than a direct effect on mediator release.

There is less information about the influence of corticosteroids on mast cell cytokine production. Corticosteroids inhibit synthesis of a number of cytokines by T lymphocytes in vitro, but their effects on mast cell cytokine production
have not yet been extensively investigated. However, preliminary evidence indicates that TNF-α synthesis and release by murine mast cells are both inhibited in vitro by dexamethasone, and that this inhibition is likely at least in part to account for the reduction in the cutaneous late phase inflammatory response observed in mice following pretreatment with dexamethasone (116). In asthma a significant reduction in BAL fluid cells expressing mRNA for IL-4 and IL-5 has been demonstrated following a short course of oral prednisolone (117), but as discussed above the proportion of mast cells in BAL fluid is very small. The effects of steroids on cytokine mRNA and protein expression in biopsy specimens from asthmatic subjects have not yet been reported.

The potent anti-inflammatory agent cyclosporin has been shown to have some beneficial action in asthma and may be useful in patients whose condition is not fully controlled with corticosteroids or who are experiencing an unacceptable level of side effects (118). Cyclosporin acts by binding to a cytoplasmic receptor, cyclophilin, and the complex then inhibits the transcriptional activation of a number of cytokine genes. Although its effects have been most widely explored in relation to T lymphocytes, recent evidence indicates that both cyclosporin and the related immunosuppressive drug FK506 inhibit production of several cytokines, including IL-2, IL-3, IL-4 and GM-CSF by murine mast cell lines (119). Cyclosporin can also partially inhibit release of histamine and PGD2 from human lung mast cells (120), and so these observations allow speculation that its anti-inflammatory action may in part be mediated by an effect on mast cells.

CONCLUSION

The recognition that mast cells can serve as an important source of cytokines has greatly extended our view of their potential role in the pathogenesis of asthma. Rather than releasing mediators that account only for the immediate bronchoconstrictor reaction to allergen challenge, it now seems probable that mast cells contribute both to the initiation and to the maintenance of the inflammatory response via the synthesis and release of cytokines. Figure 3 summarizes the possible ways in which mast cell cytokines may be implicated in the regulation of allergic inflammation.

Furthermore, the findings in relation to murine mast cells suggest that the profile of human mast cell cytokine production is likely to be substantially wider than currently recognized, perhaps including fibrogenic cytokines and other members of the chemokine family. It is also interesting that murine mast cells are able to produce not only ‘Th2-like’ cytokines such as IL-3, IL-4, IL-5 and GM-CSF, but additionally IL-2 and IFN-γ, which are more usually associated with Th1 responses and delayed-type hypersensitivity. Indeed, mast cells have been linked to the development of delayed-type hypersensitivity responses (121,122) and such responses are reduced in mast cell deficient W/Wv mice (123). These observations raise the possibility that the profile of mast cell cytokine production might vary in different situations, as is recognized to be the case with T lymphocyte cytokine profiles.

In discussions of the cellular and molecular basis of asthma, there has not infrequently been a tendency to focus on one cell type in preference to others as being of paramount importance. However, it is becoming increasingly apparent that a great variety of cells, both inflammatory and structural, are implicated in the inflammatory response underlying asthma, and that mast cells must be added to the list of potential cells involved together with T lymphocytes, macrophages, bronchial epithelial cells, eosinophils and fibroblasts. Critical to the future understanding of the induction and resolution of the airway inflammatory process will be an appreciation of the mechanisms of control of cytokine synthesis and release and the similarities and differences in these respects between different cell populations.

ACKNOWLEDGEMENTS: This work was supported by the Medical Research Council (UK).
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