

REVIEW

Mast cell cultures: bench to bedside

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Introduction

The mast cell plays a prominent role in allergic reactions and is implicated in the genesis of other diseases such as pulmonary fibrosis and graft-vs-host disease. Moreover, mast cells participate in important physiological responses such as the defence against bacteria and parasites and may also contribute to the growth of solid tumours or to immune defence against them [1,2]. As regards bronchial asthma, the perception of the mast cell's role has been changed over time, and the present view states that mast cells, along with T lymphocytes and eosinophils, substantially contribute to the establishment of bronchial inflammation [3].

In 1989, several independent investigators reported that cultivated murine mast cells were able to produce an array of multiple proinflammatory cytokines *in vitro* [4–6]. Five years later, Bradding *et al.*, using immunohistochemical analysis of endobronchial biopsies, showed that mast cells were the major source of IL-4, IL-5, IL-6 and TNF α (both in normal individuals and in subjects with mild atopic asthma [7]. This work showed that studies *in vitro* can anticipate important findings in humans that are relevant to the pathogenesis of asthma.

The aim of the present article is to briefly outline the potential role of mast cell cultures as a source of information, useful for clinical investigators in allergy, respiratory medicine and other fields of research.

In vitro and *in vivo* studies on mast cells are influenced by mast cell heterogeneity

The mast cell, unlike the basophil or the eosinophil, does not leave the bone marrow as a mature cell but in the form of an immature progenitor that, although committed to the mast cell lineage, lacks the phenotype and the functions of mast cells. The progenitors released from the bone marrow enter the bloodstream and reach peripheral tissues and organs where they differentiate under the influence of local environmental factors and assume the morphological and functional characteristics of mast cells (reviewed in [8]).

Much research has been addressed to mature, differentiated mast cells present in tissues. Overall, immunocytochemical studies strongly point out the presence of a large heterogeneity in the phenotype of mast cell populations both in rodents [9,10] and humans (reviewed in [11]). A variability of mast cell phenotype — for example, the differences in the content of cellular proteases — is present in different anatomical sites of the same individual and reflects the action of the local microenvironment.

Also, a pronounced functional heterogeneity exists in mast cells, as demonstrated by *in vitro* studies performed with mast cells recovered from various tissues and organs both in rodents (lung, gut, peritoneal cavity, etc.) and humans (lung, skin, tonsil, intestine). More specifically, the responses to different activating stimuli and the pharmacological inhibition of mast cell activation vary among cell populations derived from different tissues of the same individual or from individuals of different species.

Therefore, the study of the 'end product' (i.e. the differentiated mast cells present in tissues) does not easily render a comprehensive view of the role of the mast cell in a more general context.

Mast cell cultures: from the mouse to the man

In 1981, five independent groups reported that normal mouse bone marrow, cultivated in a medium containing particular growth factors, can generate a uniform population of cells with the feature of immature mast cells. The most important of such growth factors (in this murine system) was later identified as IL-3. In a recent article, Galli has exhaustively reviewed the 'historical' steps that led to the establishment of these normal (non-transformed) murine mast cell cultures and how they contributed to elucidate fundamental biological questions related to mast cell origin and development [12]. Therefore we refer the reader to this article for more details.

We would like to underscore that studies with mouse bone marrow-derived mast cells (BMMC) helped to clarify three important points: mast cell precursors are located in the bone marrow; IL-3 is the crucial factor for mast cell differentiation in the mouse; and mast cell phenotype can vary according to environmental stimuli. The latter

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phenomenon was elegantly demonstrated with the injection of BMMC (whose phenotype in liquid cultures is similar to mucosal mast cells of the airways or gastric mucosa) in genetically mast cell-deficient mice (W/W^v), which are virtually devoid of mast cells in tissues. The interesting results were that mast cells of donor origin assumed the characteristics of the connective tissue-type mast cell when they homed in the skin or in the peritoneal cavity [13].

Parallel studies identified another soluble factor strictly involved in the biology of the mast cell, i.e. the *c-kit* ligand or stem cell factor (SCF) — mainly produced by fibroblasts, but also from endothelial cells and keratinocytes — which interacts with the *c-kit* receptor present on mast cells and mast cell precursors. The numerous activities of stem cell factor have been previously reviewed [14]. This factor permits the mast cell to survive and develop in tissues and also regulates many functions of mature mast cells. Mast cell precursors are characterized by the presence of *c-kit* receptor, while in W/W^v mice the *c-kit* receptor is absent. In BMMC, SCF prevents the apoptosis induced by IL-3 deprivation [15] and promotes the release of IL-6, TNF α and other mediators [16].

The studies on stem cell factor in the murine system opened the way to human primary mast cell cultures. In fact, in recent years SCF was shown to be a major growth and differentiation factor for human mast cell progenitors in cord blood mononuclear cells [17], bone marrow [18,19] and fetal liver [20]. However, in order to fully obtain differentiated human mast cells, co-culture with murine skin fibroblasts [21] or additional growth factors (IL-6, prostaglandin E₂) [22] may be needed.

IL-3 does not bind to human lung mast cells [23], although it may support SCF-induced human mast cell differentiation [18,20], acting probably on SCF-responsive progenitors [24]. Recently it has been reported that IL-3 prolongs the survival of cultured human mast cells that appear to bear receptors for this cytokine [25]. Interestingly, IL-3 alone — which promotes the growth of murine mast cells — is a specific growth factor for human basophils [26–28]. Thus, in humans, IL-3 and SCF originate basophils and mast cells, respectively, acting on haemopoietic undifferentiated precursors (CD 34⁺) (Fig. 1). Mature mast cells still retain the receptor for SCF and basophils circulating in parallel display a high affinity receptor for IL-3 [27], indicating that these specific growth factors not only act during differentiation but also regulate the functions of mature cells.

Mast cell cultures: are they useful to fill the gap between basic science and clinical research?

Non-transformed mast cell cultures provide a large number of normal mast cells and so they facilitate basic research.

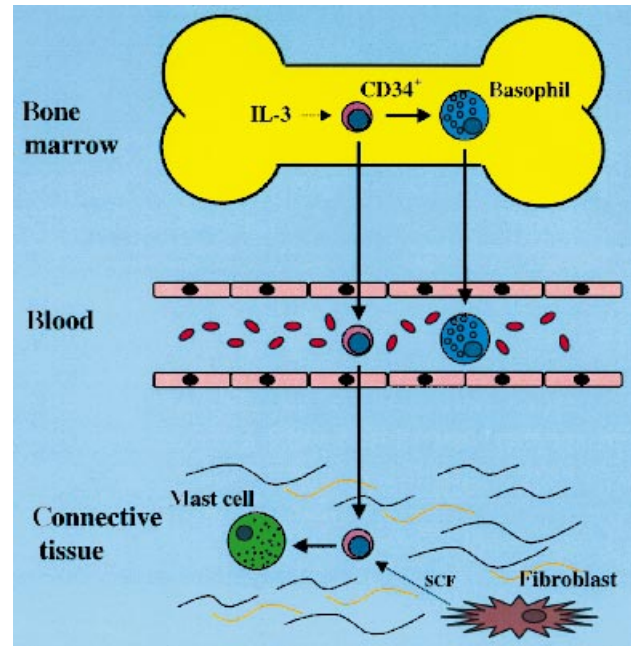


Fig. 1. Differentiation of human mast cells and basophils. Basophils differentiate in the bone marrow from multilineage hemopoietic progenitors (CD34⁺) under the influence of interleukin-3 (IL-3). Committed mast cell progenitors migrate to peripheral connective tissues where, under the influence of stem cell factor (SCF) — produced by fibroblasts — differentiate into mature mast cells.

For example, the mechanisms of mast cell activation and signal transduction have been extensively investigated, and very recent reports indicate that BMMC can still provide important information on specific features, such as the co-stimulatory receptors involved in cell activation [29], signal transduction in genetically manipulated cells [30] or the inhibition of cell activation [31]. Moreover, mast cell cultures enable us to study the response to various activating stimuli during differentiation, even in very early stages when cells have not yet acquired degranulation capacity [32]. This longitudinal approach can also be applied to examine the morphological characteristics of developing mast cells [21].

It is far beyond the aim of this paper to discuss in detail the contribution of mast cell cultures to the advancement of the knowledge of mast cell biology that has been accomplished in recent years. We would only like to expand a few points that are relevant to the pathogenesis of human diseases and that should also be of interest to the clinical investigator.

Allergic inflammation—bronchial asthma—immune defences

As mentioned above, the production of a variety of

cytokines by mast cells was first demonstrated in cultivated murine mast cells [4–6]. This observation changed the perspective of the role of the mast cell in allergic diseases and in bronchial asthma, which until that time, was thought to be strictly linked to degranulation and release of soluble chemical mediators. The capacity of the mast cell to produce and release proinflammatory cytokines (IL-4, IL-5, IL-6, IL-13, TNF α) was later confirmed *in vivo* in humans [7,33], opening a debate on the respective role of mast cells, granulocytes (eosinophils, basophils) and T lymphocytes in orchestrating allergic inflammation. Mast cell-derived cytokines, particularly IL-4 and TNF α , are implicated in recruitment of leucocytes in asthmatic airways, through the induction of VCAM-1 — the natural ligand of VLA-4 integrin expressed on activated eosinophils — on vascular endothelial cells [34] (Fig. 2a). Many questions still remain. For example, what are the local conditions that facilitate the homing of circulating mast cell progenitors in specific locations in the airways, skin, intestine, etc.? Do immature mast cells have any functional activity (for example the production of cytokines) prior to the acquisition of a fully differentiated phenotype? How do the cytokines produced by T lymphocytes influence the local development of human mast cells and, vice versa, how do mast cell-derived cytokines contribute to the polarization of T-cell responses (i.e. the acquisition of a TH₂ phenotype)? And finally, what is the role of the cytokines produced by eosinophils and basophils after their recruitment into tissues from circulation?

The availability of human mast cell cultures could help to address some of these questions. In the first place, the development of mast cells in cultures should facilitate the identification of a specific marker for human mast cell progenitors, thus complementing the efforts to locate progenitors in tissues [35]. Secondly, the study of the expression of adhesion molecules during mast cell differentiation [36], as well as of the factors that stimulate mast cell migration and chemotaxis [37,38], may shed light on the mechanisms of mast cell homing in tissues. Thirdly, a further advantage of mast cell cultures is the possibility they provide to create different microenvironments *in vitro*, using appropriate soluble growth factors, cellular co-cultures and various matrix proteins [39,40]. These cultural techniques, if applied also to human mast cells, should allow for rebuilding the conditions that lead to the acquisition of a definite mast cell phenotype *in vivo*. Lastly, human mast cell cultures are suitable for determining the production of cytokines from cells that were not subjected to prior *in vivo* activation like those isolated from tissues [41].

Mast cell cultures have also proven to be of value for the investigation of the mutual relationships between mast cells and lymphocytes. More specifically, the findings that BMMC express major histocompatibility complex (MHC)

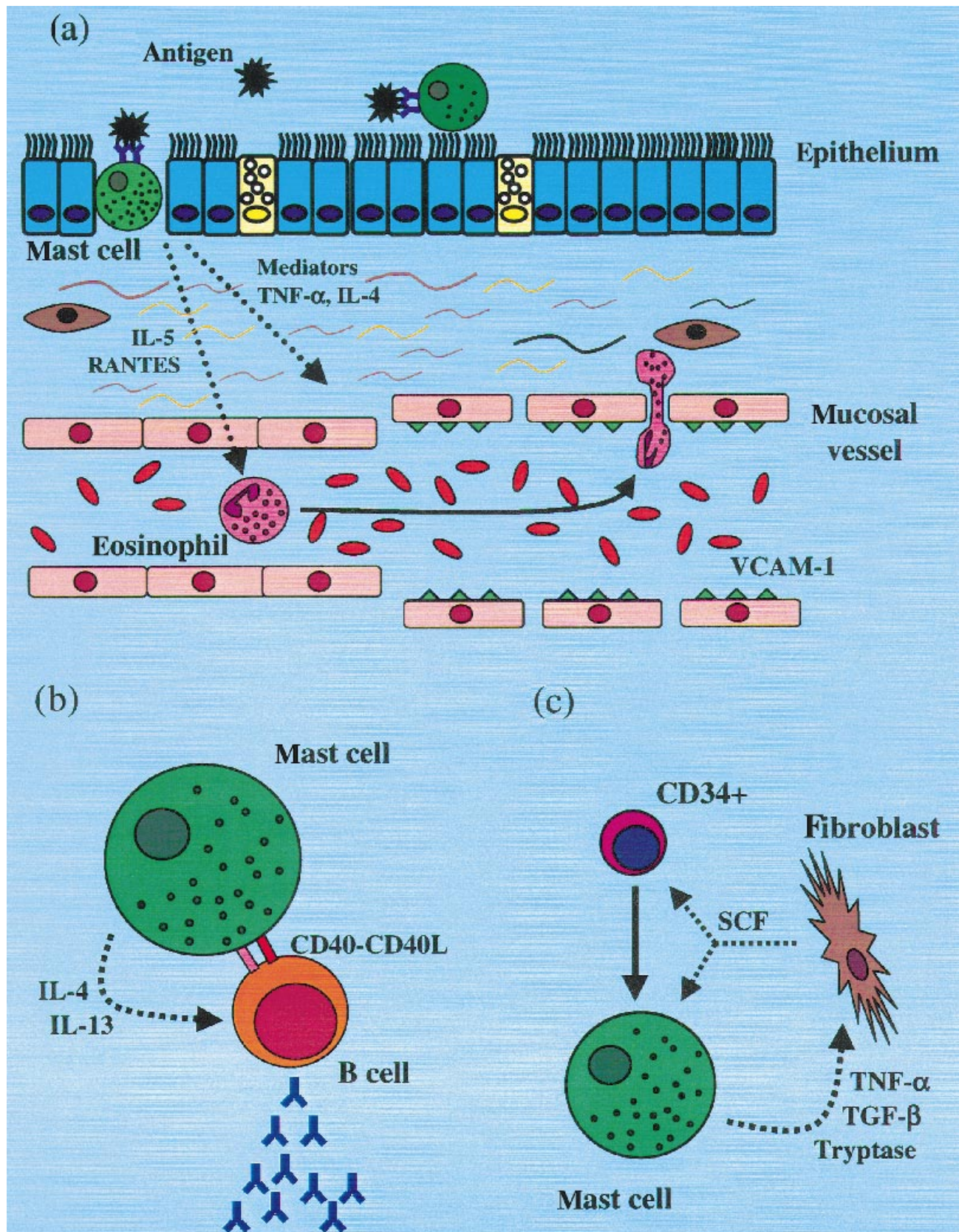
class II antigens and are able to present immunogenic peptides to CD4 T lymphocytes [42] suggest a role of mast cells as antigen-presenting cells (APCs) implicated in the activation of antigen-specific T-cell clones. This view is strengthened by the recent observation that BMMC express the CD80 and CD86 molecules [43], and therefore can also provide co-stimulation to T lymphocytes. In addition, BMMC can interact with B lymphocytes. In fact, they induce T-cell-independent B-cell activation and antibody production, through the secretion of a soluble factor named mast cell-derived B-cell-stimulating activity (MC-BSA) [44]. This observation is consistent with the finding that purified human lung mast cells and blood basophils [45], as well as nasal mast cells of allergic patients [46], can directly induce IgE synthesis in B cells, through the expression of CD40 ligand and the production of IL-4 and IL-13.

Overall, these experimental findings strongly suggest that mast cells co-operate with T and B lymphocytes in the immune network. Particularly, the co-operation with T cells, along with the release of mediators triggered by non-immunological stimuli or by IgE receptors cross-linking, may be relevant to host defence against bacterial infections and parasites. Indeed, it has been recently reported that mast cell-deficient mice appear to have impaired defences against experimental septic peritonitis [47]. On the other hand, mast cells may function as a surrogate of T helper cells when they interact directly with B cells (Fig. 2b). This phenomenon could play a significant role in the production of IgE outside the lymphoid tissues, for example in the nasal or bronchial mucosa, locally amplifying allergic inflammation in conditions such as allergic rhinitis and bronchial asthma.

Fibrotic disorders and other diseases

In the past, histopathological studies established that the number of mast cells is increased in the early stages of

Fig. 2. Mast cell interactions with eosinophil, B lymphocyte and fibroblast. Mucosal mast cells (MC) are activated by allergens which, upon IgE cross-linking, induce MC degranulation of stored and newly synthesized substances (a). MC mediators (histamine, prostaglandin D₂, leukotriene C₄, etc.) act on mucosal vessels, causing vasodilatation and increased vascular permeability. MC-derived cytokines (TNF α , IL-4) induce the expression of adhesion molecules (VCAM-1) on vascular endothelial cells, facilitating diapedesis of leucocytes (eosinophils), previously recruited from circulation by chemotactic factors also originating from MC itself (IL-5). MC may co-operate with B-lymphocyte (b) in mucosal tissue, providing the appropriate stimuli (IL-4, IL-13, CD40, CD40L) for local production of IgE. MC proteases (tryptase) and cytokines (TNF α , TGF β) can induce fibroblast activation and proliferation (c); in turn fibroblasts, through SCF, promote maturation of MC progenitors and activation of mature MC.



fibrosis and in areas of tissue repair and remodelling, particularly around blood vessels. This observation suggests a role for the mast cell in the pathogenesis of conditions such as fibrotic lung disorders, graft vs host disease, scleroderma and wound healing [48]. Clearly, the pattern of mast cell activation in fibrosis — a chronic process — is different from that of immediate allergic reactions. Accordingly, a peculiar type of mast cell degranulation (piecemeal degranulation) has been described [49], characterized by the retention of partially filled and empty granules, distinct from classic anaphylactic degranulation characterized by granule extrusion. In this regard, very recently Dvorak and Morgan have documented the secretion of histamine *in vivo* during piecemeal degranulation of human mast cells [50], suggesting that the same mediator can be secreted by the mast cell with different mechanisms. Although the nature of mast cell activators that take part in fibrotic diseases has not been determined, it has been speculated that chronic mast cell activation is caused by the constant presence of endogenous stimuli in contrast with immediate allergic activation caused by exposure to exogenous allergens [48]. Interestingly, the number of eosinophils can also be augmented in fibrotic disorders and several *in vitro* studies have tried to unravel the complex interactions between mast cells, eosinophils and fibroblasts [48]. A recent study reported that tryptase stimulates both the synthesis of procollagen mRNA and chemotaxis in fibroblasts [51] and that BMMC promote fibroblast proliferation through the release of TNF α and TGF β [52] (Fig 2c). These findings indicate that our understanding of the cellular and molecular mechanisms of fibrotic diseases is progressing and that mast cell cultures can substantially contribute to this interesting field of research.

Similarly, the availability of a large number of non-transformed mast cells (especially of human origin) may facilitate the comprehension of the role of the mast cell in a growing list of diseases in which this multifunctional effector cell has been implicated. While the efforts of many basic and clinical investigators still continue to elucidate the pathogenesis of classical, widespread immunological disorders such as chronic urticaria and atopic dermatitis, others are evaluating the contribution of mast cells to inflammatory bowel diseases (Crohn's disease and ulcerative colitis) [53] and rheumatoid arthritis [54]. Moreover, the interactions between mast cells and nerves have been actively investigated [55,56], as has also been the role of human heart mast cells in atherosclerosis and anaphylaxis [57]. As regards cardiac mast cells, an original procedure for their isolation and partial purification from human hearts has been recently developed, thus facilitating the study of the functions of these cells *in vitro* [57].

A recent study reports that human mast cells stimulate vascular tube formation by secreting tryptase, which acts as

a potent and previously unrecognized angiogenic factor [58]. This observation is relevant to previous studies indicating the accumulation of mast cells and basophils around tumours [59], as well as a possible role of mast cells in tumour-associated angiogenesis — an essential requirement for the growth of solid tumours [60] — and tumour proliferation [61]. Hopefully, future studies will ascertain whether the increased density of mast cells in tumours indicates host defence or growth promotion.

Lastly, study of the action of the various growth factors and cytokines that affect mast cell differentiation and function (that is clearly facilitated by the developing of mast cells in culture) is essential for the comprehension of the pathogenetic mechanisms that underlie the different forms of systemic mastocytosis, varying from mild hyperplasia to fatal mast-cell leukaemia [62].

Mast cell cultures and pharmacological research

The usefulness of mast cell cultures in pharmacological studies derives from their ease of producing large numbers of cells, thus this technique has been widely exploited. The activity of many anti-allergic drugs has been tested in experimental systems based on the IgE-mediated and non-IgE-mediated activation of BMMC.

Since the purpose of this paper does not include an extensive review of the large body of literature concerning pharmacological investigations with cultured mast cells, we would like to focus on selected studies performed with corticosteroids and cromones and to briefly outline potential future applications of mast cell cultures in pharmacological and toxicological research.

Corticosteroids and mast cells

Although glucocorticoids (GC) may inhibit mediators release from murine mast cells [63,64], they do not appear to have a direct inhibitory effect on human lung mast cells [65]. However, it has recently been shown that GC exert other effects on mast cells that may be relevant to their therapeutic action in bronchial asthma and allergic diseases. More specifically, dexamethasone inhibits the production of TNF α by BMMC *in vitro* [66,67], as well as the TNF α -dependent inflammation in mice *in vivo* [66]. Moreover, in BMMC, glucocorticoids inhibit the SCF- and IL-3-induced proliferation [67], the IL-10-mediated expression of chymases [67] and the IgE aggregation-dependent expression of lymphotactin (a lymphocyte-specific chemokine) mRNA [68].

Taken together, these findings suggest that GC regulate the activity of mast cells at different levels, independently from the classical IgE-mediated degranulation and mediators release. In addition, the recent report by Finotto *et al.*

that GC down-regulate SCF mRNA expression and SCF protein production in fibroblasts helps to clarify, at the molecular level, the mechanism by which corticosteroids reduce tissue mast cells number [69], a phenomenon that is strictly relevant to the effectiveness of long-term treatment with inhaled steroids in asthma.

In the near future, human mast cell cultures will probably facilitate the understanding of the action of glucocorticoids on human mast cells. Accordingly, preliminary studies suggest that dexamethasone inhibits the production of cytokines, but not the release of histamine from cultured human mast cells [70]. Further studies will clarify whether the inhibition of cytokine production in mast cells has a therapeutic relevance in comparison to the well-known GC-dependent inhibition of cytokine production in lymphocytes and eosinophils.

Cromones and mast cells

Despite the large use of the so called 'mast cell stabilizers' in the treatment of respiratory and gastro-intestinal allergy, their mechanism of action has not yet been completely understood. Previous studies indicated that sodium cromoglycate inhibits allergen-induced histamine release from rat peritoneal mast cells [71] but not from intestinal mucosal mast cells [72]. In BMMC a global inhibition of mediators release appears only after chronic exposure (at least 5 days) to sodium cromoglycate [73]. Further studies demonstrated that high concentrations of sodium cromoglycate and nedocromil sodium are required to achieve even a modest inhibition of mediator release *in vitro* from human mast cells isolated from different tissues, and also that skin, lung, tonsillar, adenoidal and intestinal mast cells largely vary their sensitivity to cromones [74,75].

Overall, the data available suggest that the inhibition of mediators release from mast cells may not be the main pharmacological action of cromones. Other mechanisms, for example the inhibition of sensory nerves, may be more relevant to their therapeutic effects or, alternatively, their action on mast cells may involve other cellular functions, such as the release of cytokines [76]. Additional studies are needed to better clarify the action of cromones on the various aspects of mast cell activation.

Potential future applications

Mast cell cultures provide an ideal opportunity to confirm the putative anti-allergic properties that have been claimed for several drugs, for example the new antihistamines [77]. Moreover, human mast cell cultures may facilitate the testing of many drugs and substances that display mast cell degranulating effects, such as muscle relaxants, general anaesthetics, opioids, benzodiazepines and radiocontrast media [78].

This same method could be also applied to the screening of the numerous new chemicals and potentially allergenic substances that are continually introduced in the environment. Additionally, *in vitro* screening might be easier, more reliable and also more ethical when compared to traditional *in vivo* animal models.

Finally, mast cell cultures may contribute to ascertain whether air pollutants exert any direct effect on mast cells. This topic is relevant to the epidemiological evidence of increased prevalence and incidence of allergic diseases in western countries. Although the assessment of the effects of air pollutants on cultured mast cells entails considerable technical difficulties, by adapting the experimental models described in published experiments regarding the effects of air pollutants on several types of cultured cells, including human fibroblasts, B-lymphocytes, alveolar macrophages and epithelial cells [79] similar investigations in mast cells might be facilitated.

Conclusions

The methods for obtaining pure populations of murine mast cells have been established for many years. More recently, human mast cells have also been grown in culture. The development of mast cells in cultures is particularly important when considering the peculiar differentiation of the mast cell in tissues when compared to other inflammatory cells such as the neutrophil, the eosinophil and the basophil that leave the bone marrow as fully differentiated cells. Indeed, mast cells mature in peripheral tissues, where their progenitors are subjected to the influence of a variety of micro-environmental factors that, at least in part, can be reproduced *in vitro*. Furthermore, mast cell cultures yield a large number of cells, while the isolation and purification of mast cells from tissues is sometimes a difficult procedure.

Until now, mast cell cultures have been mainly used by basic investigators and, consequently, they have been of fundamental importance in clarifying many aspects of mast cell biology. We think that the time has also come for clinical investigators who are motivated in pursuing high quality research to take advantage from this *in vitro* model. In fact, mast cell cultures are suitable for the evaluation of drugs, both mast cell stabilizers and mast cell activators, provided that researchers devote the necessary time and effort to acquire familiarity with the techniques of cell culture and mediator release assays. Moreover, cultures may help complement observations that originate from studies *in vivo* (for example histopathological findings of bronchial biopsies) focusing on functional aspects. For the large majority of clinical investigators in allergy and respiratory medicine, the collaboration with basic researchers will hopefully furnish cultural stimuli that facilitate a better comprehension of disease mechanisms,

particularly but not exclusively allergic disorders, bronchial asthma and fibrosis. In this regard, mast cell cultures have already proven to be instrumental.

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