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MAST CELLS BEYOND ALLERGY: THEIR ROLE IN FIBROTIC CONDITIONS

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SUMMARY

• Mast cells play a central role not only in type I hypersensitivity reactions, but also in chronic inflammatory processes resulting in fibrosis. Fibrosis is a process characterized by fibrob last proliferation and/or by excessive production and deposition of collagen and other extracellular matrix components. The close proximity of mast cells andfibroblasts in the connective tissue enables the interaction between these two cell types. Fibroblasts have been shown to provide the microenvironmentfor connective tissue mast cell differentiation and survival. On the other hand, mast cells can affect fibroblasts through the release of various mediators with either fibrogenic or fibrolytic activities. Mast cells were shown to be present in active form in various fibrotic conditions such as scleroderma, chronic graft-versus-host disease, eosinophilic fasciitis, wound healing, idiopathic pulmonary fibrosis, and ocular cicatricial pemphigoid. This review presents the current data about mast cell and these fibrotic disorders.

INTRODUCTION

• Mast cells (MC) are tissue dwelling cells which contain prominent cytoplasmic granules that characteristically stain metachromatically with cationic dyes and possess surface receptors for diferent ligands, including irnmunoglobulin E (IgE). These cells are widely distributed in the body, being found in skin connective tissue and in *tunica serosa* and *tunica mucosa* of different organs. Mast cells play a central role in eliciting type I hypersensitivity reactions, but they have also been associated with autoimmune diseases, cancer and reactions against parasites (1).

A growing body of data that has accumulated during the last years has indicated that MC also have a central role in chronic inflammatory processes with different etiopathologies, often resulting in fibrosis.

The presence of MC in the early stages of fibrosis in areas of tissue remodeling and repair was first observed by Paul Ehrlich in 1878, who hypothesized a role for MC in fibrosis (2). Since then, MC were proposed to participate in various fibrotic conditions such as scleroderma, chronic graft-*versus-host* disease (cGVHD), eosinophilic fasciitis, wound healing, idiopathic pulmonary fibrosis and ocular cicatricial pemphigoid (3-6).

The hypothesis of a role of MC in fibrosis was strengthened since the recent observation that MC can produce cytokines (1, 7) apart from the classical preformed mediators such as histamine, proteoglycans, proteolytic enzymes, and newly formed mediators such as prostaglandins, leukotriens and platelet activating factor (1). It was also shown that MC can be stimulated by cytokines and histamine-releasing factors in a chronic and slow fashion, which is probably characteristic of chronic inflammatory processes rather than acute anaphylactic ones (8-9). The MC ability in normal and pathologic tissues to produce

type VIII collagen further supports their role in fibrosis (10). Fibroblasts and myofibroblasts are the key cells in tissue remodeling. The close proximity of MC and fibroblasts in connective tissues enables the interaction between these two cell types. Fibroblasts have been shown to provide the microenvironment for MC differentiation and survival. On the other hand, MC can affect fibroblasts through the release of various mediators with either fibrogenic or fibrolytic activities. Histamine, for example, can stimulate proliferation of skin fibroblasts, and secretion of procollagen by these cells (11). The serine protease chymase secreted by MC can activate procollagenase and cause connective tissue degradation (12).

Fibrotic conditions are diseases characterized by increased fibroblast proliferation and enhanced secretion of procollagen and extracellular matrix components. As a result, morphological and functional changes in tissues in various target organs appear. Examples of such disorders include scleroderma, cGVHD, eosinophilic fasciitis, atherosclerosis, ocular cicatricial pemphigoid, neurofibromatosis, rheumatoid arthritis, and wound healing and its abnormal manifestations, such as hypertrophic scars andkeloids (13-17). The number of MC infibrotic diseases increases during the active inflammatory process, but decreases as the involved tissue turns into scar tissue. Although MC may be of a major importance in fibrosis, it should be kept in mind that the information gathered reflects only a small part of a more complex system in which many other components, in addition to MC and fibroblasts, interact. Other immune cells can, for example, influence fibroblast activity by secreting fibrogenic cytokines that stimulate fibroblast proliferation and collagen production. This review presents current data about MC in some fibrotic conditions (Table 1), focusing on the possible role of MC in fibroblast activation.

SCLERODERMA

• Scleroderma is an autoimmune disease of unknown etiology. It is characterized by diffuse fibrosis in the skin alone *(morphea),* or also in internal organs (systemic sclerosis).

Table 1. Mast cell-related fibrotic conditions

Fibrotic conditions	Refs
Scleroderma	3, 13, 19-25
Chronic graft-*versus*-host disease	6, 29-31
Eosinophilic fasciitis	4, 33, 34
Wound healing	5, 36-40
Idiopathic pulmonary fibrosis	13, 41-48
Ocular cicatricial pemphigoid	14, 15

Excessive secretion of procollagen type I and fibronectin was found in these patients (18). The role of MC in scleroderma was addressed in several studies (19 and Refs therein). Increased MC number was observed in the early inflammatory stage of the disease, neither in areas of uninvolved skin, nor in the late quiescent stage (20). Another study had demonstrated increased releasability of MC granules in affected skin in scleroderma (21). An increase in MC number and degranulation was found to precede the clinical signs of skin fibrosis in systemic sclerosis (22). Fibrogenic cytokines secreted from MC that may contribute to fibrosis in scleroderma are tumor necrosis factor-oc and interleukin-4 (IL-4). The latter was detected more frequently in sera of scleroderma patients than in controls (23).

The possible role of MC in fibrosis has led to clinical trials using ketotifen, an inhibitor of MC mediator release, as a treatment for scleroderma (16). Although one study reported a beneficial effect of this drug in two patients with scleroderma (24), another, double-blind placebo-controlled clinical trial of ketotifen in 24 patients failed to demonstrate a significant improvement (25).

CHRONIC GRAFT-versus-HOST DISEASE

• This condition is a serious complication of allogenic bone-marrow transplantation occurring 3 to 12 months after transplantation in up to 30% of patients. It is characterized by collagen deposition in various target organs, production of autoantibodies, and immunodeficiency. The fibrotic processes cause scleroderma-like skin changes, pulmonary fibrosis, gastrointestinal malabsorption, and severe keratoconjunctivitis sicca (26).

A murine model of cGVHD was developed by intravenous injection of B10/D2 spleen cells to sublethally irradiated Balb/c mice (27). This model was obtained across minor histocompatibility barriers and resembled the human disease. In fact, several weeks following injection, the histopathologic findings of dermal fibrosis, mononuclear infiltrate and loss of dermal fat resembled human cGVHD (28). In this model, connective tissue MC from kidney capsule, tongue, and peritoneal cavity were found to be completely degranulated just before fibrosis developed: a striking decrease of MC number was demonstrated from day 12 onwards, and a complete disappearance from day 20 to day 120 when fibrosis was evident (29). Later on, MC reappeared and increased in numbers as the fibrotic processes gradually subsided. Therefore, it seems that a temporal relation between MC activation and fibrosis appearance exists in this cGVHD model.

To investigate the precise role of MC in cGVHD and to reveal a source of activating factors in this disease, the effect of spleno-

cytes from cGVHD mice on peritoneal MC was evaluated. Supernatants of these splenocytes induced MC activation manifested by a slow histamine release after 4-5 days of incubation. This activation was evident when the splenocytes were from early stage cGVHD mice with extensive fibrosis but not with mice whose cGVHD and fibrotic manifestations had resolved (30). Similar observations were made also in human cGVHD. In fact, supernatants of peripheral blood mononuclear cells from cGVHD patients displayed a histamine-releasing activity towards rat peritoneal MC (31). Also, this MC activation had a slow onset and was continuous. MC histamine release was partially inhibited by the addition of an anti-IL-1 neutralizing antibody, indicating that IL-1 is one of the histamine releasing factors contained in the supernatant (31, see also 8).

In a recent study on cGVHD patients, a fivefold decrease in MC number from biopsies of involved skin compared to controls was found. This observation suggests that MC have degranulated and contributed to the fibrotic process. Treatment of these patients with ketotifen for 3 months normalized MC count and correlated well with a general clinical improvement (32). In animal model, nedocromil sodium, an antiallergic drug with MC stabilizing properties, was administered to cGVHD mice for 18 days, beginning three days before the induction of the disease (6). Another group of mice was injected with compound 48/80 to activate MC. Nedocromil treatment normalized both peritoneal and skin MC number with a concomitant improvement of skin manifestations. On the other hand, compound 48/80 caused a complete disappearance of toluidine blue stainable peritoneal and skin MC, and induced skin changes resembling mild cGVHD (6).

The above studies from both human disease and murine model demonstrate the important role of MC activation in the fibrotic process of cGVHD and clearly indicate that MC stabilization may be therapeutic target in this disease.

EOSINOPHILIC FASCIITIS

• Eosinophilic fasciitis (EF) is characterized by fibrosis of the middle parts of the limbs, sometimes extending to the trunk. Skin fibroblasts change into an activated form and produce excessive collagen amounts (4). The hypothesis is that activated MC attract eosinophils by secreting eosinophil chemotactic factors and the eosinophils later on affect fibroblasts. However, studies have shown that MC may also act directly in promoting fibrosis in this disease. Infiltration consisted of MC and increased histamine levels were found in tissues in early stages of EF (33). Several reports have demonstrated the efficacy of cimetidine, a histamine (H,)-receptor antagonist, in the treatment of EF (34). The effect of this drug was probably a result of inhibition of MC degranulation and of antagonism towards the fibrogenic effects of histamine.

WOUND HEALING

• Wound healing (WH) is a complex process that starts immediately after injury and consists of inflammation, tissue granulation, and matrix formation (35). Following injury, there is initial increase in MC number around blood vessels followed by a plateau and return to baseline levels. MC participate in all stages of WH and affect fibroblasts mainly during the phase of matrix formation.

Activity of MC in WH was evaluated in a rat model of incisional wounding, following the use of compound 48/80 to activate MC. An increased wound breaking strength and increased collagen deposition was detected following treatment with compound 48/80 (36). In another rat model of perforated mesentery, compound 48/80 significantly improved wound healing. However, this effect was not changed after addition of H,-receptor antagonist, implying that histamine was not responsible for the improvement in healing (37). On the other hand, another study has demonstrated that while low doses of histamine increase collagen formation in rat skin wounds, high doses decreased it (38). In an *in vitro* model of wound consisting of MC co-cultured on 3T3 fibroblast monolayer, a "wound" was performed by scraping away half of the monolayer. Activation of MC with compound 48/80 or with anti-IgE antibodies increased fibroblast migration and proliferation into the cell free area (39). Histamine was observed to be partially responsible for the MC enhancing effect on fibroblast migration and proliferation in this *in vitro* model (40). This may also be the case in keloids (11).

IDIOPATHIC PULMONARY FIBROSIS (FIBROSING ALVEOL1TIS)

• This lung disorder is manifested by inflammation and fibrosis of pulmonary interstitium and peripheral airspaces (41). It may be associated with asbestos inhalation, drugs (amiodarone, gold, busulphan, bleomycin) or with other chronic diseases, such as rheumatoid arthritis and scleroderma..In lung biopsies of patients with this disease, MC were found in fibrotic areas (42). High levels of histamine and tryptase were also detected in bronchoalveolar lavage (BAL) fluid (43), indicative of MC activation.

Animal studies in which pulmonary fibrosis was induced by radiation, bleomycin or asbestos were performed to assess the role of MC. In these animal models, increased MC number was always found (44-46). In a more recent study of lung fibrosis induced by radiation, captopril, an angiotensin-converting enzyme inhibitor, reduced lung fibrosis concomitantly with reducing MC number (47). As with skin fibrotic diseases, it may be that a chronic low-grade MC activation is responsible for pulmonary fibrosis. A recent study has demonstrated the pre-

sence of histamine-releasing factor in B AL fluid of patients with pulmonary fibrosis, which caused the *in vitro* release of histamine, p-hexosaminidase and leukotriene C4 from MC (48).

OCULAR CICATRICIAL PEMPHIGOID

• Ocular cicatricial pemphigoid (OCP) is a chronic inflammatory disease of presumed autoimmune etiology. It is characterized by chronic cicatrizing conjunctivitis and progressive conjunctival subepithelial fibrosis, which result in fornix shortening, symblepharon formation, trichiasis, distichiasis, meibomian duct obstruction and reduced tear supply to the ocular surface (14,15). The disease is bilateral and eventually results in blindness in a high proportion of these patients due to corneal scarring. No topical treatment is effective in stopping conjunctival scarring. High doses of systemic corticosteroids, alone or in combination with systemic immunosuppressive therapy, are required to control the disease. One of the histopathological findings in the conjunctiva is considerable MC presence in the substantia propria inflammatory cell infiltrate (14, 15). Furthermore, the ratio of connective tissue MC to mucosal MC was significantly higher in active and advanced OCP patients when compared with normal controls or with patients with a mild form of the disease (15). This observation is interesting since connective tissue MC have been implicated in other fibrotic diseases discussed herein. In this regard, recent data of involvement of MC and nerve growth factor in vernal conjunctivitis are also provocative (49, see also 13, 19).

CONCLUSION

The data gathered from *in vivo* studies on patients and from animal models show that MC may play an important role in fibrosis. *In vitro* studies have shown that MC mediators have the capacity to directly affect fibroblast migration, proliferation and secretion. Further studies are needed to find the specific MC activators which act in fibrosis and to understand the interaction between MC and fibroblasts under various pathological conditions. Thus MC could represent a novel target for the development of therapeutic strategies to treat fibrotic disease (13, 16, 24, 25, 32, 34, 47).

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