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Mast Cells and Fibroblasts Work in Concert to Aggravate Pulmonary Fibrosis

Role of Transmembrane SCF and the PAR-2/PKC-α/Raf-1/p44/42 Signaling Pathway

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Mast cell (MC) accumulation has been demonstrated in the lungs of idiopathic pulmonary fibrosis (IPF) patients. Mediators released from MCs may regulate tissue remodeling processes, thereby contributing to IPF pathogenesis. We investigated the role of MC—fibroblast interaction in the progression of lung fibrosis. Increased numbers of activated MCs, in close proximity to fibroblast foci and alveolar type II cells, were observed in IPF lungs. Correspondingly elevated tryptase levels were detected in IPF lung tissue samples. Coculture of human lung MCs with human lung fibroblasts (HLFs) induced MC activation, as evinced by tryptase release, and stimulated HLF proliferation; IPF HLFs exhibited a significantly higher growth rate, compared with control. Tryptase stimulated HLF growth in a PAR-2/PKC-α/Raf-1/p44/42—dependent manner and potentiated extracellular matrix production, but independent of PKC-α, Raf-1, and p44/42 activities. Proproliferative properties of tryptase were attenuated by knockdown or pharmacological inhibition of PAR-2, PKC-α, Raf-1, or p44/42. Expression of transmembrane SCF, but not soluble SCF, was elevated in IPF lung tissue and in fibroblasts isolated from IPF lungs. Coculture of IPF HLFs with MCs enhanced MC survival and proliferation. These effects were cell-contact dependent and could be inhibited by application of anti-SCF antibody or CD117 inhibitor. Thus, fibroblasts and MCs appear to work in concert to perpetuate fibrotic processes and so contribute to lung fibrosis progression. *(Am J Pathol 2013, 182: 2094—2108; http://dx.doi.org/10.1016/j.ajpath.2013.02.013)*

Mast cells (MCs) originate from CD34-expressing hematopoietic stem cells in the bone marrow. They circulate in the blood as monocyte-like precursors and then home to tissues, where they mature under the influence of stem cell factor (SCF) and local cytokines.[1] MCs are predominantly localized at sites that have direct contact with the external environment, such as the skin, airways, and intestine, where they function as sentinel cells in host defense.[2] Upon activation, MCs release their granule contents, which include proteases (tryptase, chymase, carboxypeptidase), vasoactive amines (histamine, serotonin), proteoglycans (heparin, chondroitin sulfate), and growth factors [transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF)].[3] In addition, activated MCs are able to produce a variety of cytokines [IL-1α, IL-6, granulocyte-monocyte colony-stimulating factor (GM-CSF), interferon-α (IFN-α)], chemokines (CCL-2, CCL-5, CXCL-1), and lipid mediators [leukotriene C4 (LTC_4), LTB_4, prostaglandin 2 (PGE_2)].[3] The broad spectrum of

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molecules produced by MCs might explain their varied functions: the recruitment, activation, and differentiation of inflammatory cells[4–6] and the regulation of vascular permeability,[2] smooth-muscle cell contractility,[7] and fibroblast growth.[8] Thus, MCs have been found to be involved in the pathogenesis of allergic, chronic inflammatory, and fibrotic diseases.

An association between MC infiltration and the degree of fibrosis has been found in various kidney disorders, including focal segmental glomerulosclerosis, IgA nephropathy, renal amyloidosis, and lupus nephritis,[9–11] as well as in liver cirrhosis[12] and in the minor salivary glands of patients with Sjögren's syndrome.[13] In addition, a growing body of evidence suggests that MCs may play a role in the pathogenesis of fibrotic lung diseases. Elevated numbers of MCs were found in the lungs of patients with sarcoidosis,[14] cryptogenic organizing pneumonia,[15] hypersensitivity pneumonitis,[15] silicosis,[16] and idiopathic pulmonary fibrosis (IPF).[17–19] Moreover, increased levels of tryptase were measured in bronchoalveolar lavage fluid (BALF) from IPF patients, and tryptase-positive IPF cases were reported to have a poorer outcome.[20] The possible involvement of MCs in the pathogenesis of IPF may arise from their ability to produce a variety of profibrotic factors. In this regard, tryptase, the most abundant protein in human lung MC, was found to stimulate collagen I synthesis[21] and fibroblast proliferation.[8] Similar cellular activities were reported on exposure of fibroblasts to TGF-β1, bFGF, or histamine,[22] which are other mediators released from activated MC. Moreover, it was shown that both tryptase and chymase are capable of activating matrix metalloproteinases, thereby contributing to extracellular matrix (ECM) turnover.[23,24]

Although MC mediators are known to have profibrotic properties, the molecular mechanism of their action is poorly understood. The major focus of the present study was therefore to decipher the molecular basis of MC—fibroblast interplay in the development of pulmonary fibrosis.

Materials and Methods

Study Population

The investigations were conducted according to Declaration of Helsinki principles and were approved by the local institutional ethics committee. Informed consent was obtained from either the patients or their next of kin. BALF was obtained by flexible fiberoptic bronchoscopy from 20 spontaneously breathing healthy volunteers and from 40 spontaneously breathing IPF patients. Diagnosis of IPF was decided on the basis of recently published guidelines.[25] In 20 patients, diagnosis was confirmed by surgical lung biopsy, which revealed a pattern of usual interstitial pneumonia in every case. Additionally, lung tissue was obtained from 24 IPF patients who underwent lung transplantation at the Department of Cardiothoracic Surgery, Medical University of Vienna, Austria. IPF diagnosis was based on clinical criteria, as well as proof of a usual interstitial pneumonia pattern. Nonused donor lungs served as control ($n = 10$). Inflammatory processes were not observed in donor lungs by histopathological evaluation. Demographic and clinical characteristics of the patient cohort are reported elsewhere.[26]

Isolation of Human Lung MCs

For isolation of human lung MCs, tumor-free lung tissue obtained from patients with bronchial carcinoma undergoing lobectomy was provided by the Section of Pathology, Research Center Borstel. Approval for these studies was obtained from the institutional review board at the University of Lübeck, and informed consent was provided according to the Declaration of Helsinki.

Lung specimens were chopped into pieces and placed overnight in ice-cold MC buffer (12 mmol/L HEPES, pH 7.4, 290 mmol/L NaCl, 3 mmol/L KCl, 3.7 mmol/L $Na_3PO_4 \cdot 12H_2O$, 6 mmol/L glucose, 0.1% bovine serum albumin). To disperse cells enzymatically, lung pieces were incubated in MC buffer containing 0.1% gelatin, 1.5 mg/mL dispase II, 0.375 mg/mL chymopapain (both from Sigma-Aldrich, Taufkirchen, Germany; St. Louis, MO), 0.75 mg/mL collagenase type I, 1.79 mg/mL elastase (both from Worthington, Lakewood, NJ) under agitation for 4 hours at 37°C. MCs were enriched by Percoll gradient centrifugation followed by immunoaffinity magnetic enrichment, using anti-phycoerythrin—conjugated microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany; Auburn, CA) in combination with phycoerythrin-conjugated mAb 97A6 (IOTest; Beckman Coulter—Immunotech, Marseille, France; Fullerton, CA) specific for CD203c. The purity of MCs used for experiments ranged from 88% to 100% as assessed by Toluidine Blue staining. Viability was always >85% as assessed by Trypan Blue exclusion. Purified MCs (5×10^5/mL) were cultured in StemPro medium (Life Technologies—Invitrogen, Carlsbad, CA) containing 2 mmol/L L-glutamine, 50 IU/mL penicillin, 50 μg/mL streptomycin, and 100 ng/mL human recombinant SCF (PeproTech, London, UK; Rocky Hill, NJ). MCs taken from culture 5 days after isolation were washed with fetal calf serum—free medium and were seeded onto confluent serum-starved human lung fibroblasts (HLFs) at a 1:2 ratio. When required, 100 nmol/L of the CD117 inhibitor ISCK03 (Santa Cruz Biotechnology, Santa Cruz, CA), 10 μg/mL anti-human SCF (R&D Systems, Wiesbaden, Germany; Minneapolis, MN), or 10 μg/mL isotype IgG control (R&D Systems) was included. MC numbers during the coculture period were assessed using Kimura staining, which readily differentiates red metachromatic MCs from unlabeled HLFs. MC monoculture controls were established in parallel.

To investigate tryptase release, MCs were incubated with control or IPF HLFs in Dulbecco's modified Eagle's medium alone or in the presence of 2 μg/mL compound 48/80 (C48/80; Sigma-Aldrich), or were challenged with BALF obtained from healthy control subjects or IPF