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Original contribution

Transforming growth factor β– and interleukin 13–producing mast cells are associated with fibrosis in bone marrow☆

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Summary Although bone marrow fibrosis is a lethal condition, its underlying mechanism is not fully understood. This study aimed to investigate the pathogenesis of fibrosis in the bone marrow through histologic examination of mast cell infiltration and the expression of fibrosis-associated cytokines. We analyzed 22 bone marrows with fibrosis (8 primary myelofibrosis [PMF], 5 post–essential thrombocythemia [ET], myelofibrosis, and 9 myelodysplastic syndrome [MDS] with bone marrow fibrosis [BMF]). Immunohistochemical and immunofluorescence stainings were performed using anti–mast cell tryptase, interleukin (IL) 13, transforming growth factor β (TGF-β), CD34, and CD42b antibodies. The number of mast cells in bone marrows with fibrosis was significantly higher than that in controls ($P < .0001$ for all cases with fibrosis versus control, $P = .0470$ for PMF versus control, $P < .0001$ post-ET myelofibrosis versus control, and $P = .0005$ for MDS with BMF versus control). Moreover, bone marrows with higher fibrotic grades exhibited greater amounts of infiltrating mast cells. Mast cells were positive for TGF-β and IL-13 in bone marrows with fibrosis of all 3 groups. Megakaryocytes were negative for TGF-β in post-ET and MDS with BMF, but some megakaryocytes in PMF were weakly positive for TGF-β. Megakaryocytes were negative for IL-13 in all 3 groups. Blasts were negative for both TGF-β and IL-13 in all 3 groups. Thus, TGF-β– and IL-13–producing mast cells might be key players in the development of BMF. Therefore, mast cells could be potential therapeutic targets for the treatment of BMF.

1. Introduction

Bone marrow fibrosis (BMF) is characterized by the increased deposition of reticulin and collagen fibers. The current BMF scoring system is primarily based on the density and type of fibrosis [1]. Myelofibrosis (MF) can present as

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primary myelofibrosis (PMF) or arise from a preexisting diagnosis of polycythemia vera (post-PV MF) or essential thrombocythemia (post-ET MF). Myelodysplastic syndrome (MDS) is sometimes complicated with BMF, with an incidence of 11% to 17% of total MDS cases [2].

BMF is a lethal condition, which currently has no effective treatments, and its exact pathogenesis is not fully understood. Recently, the role of increased JAK–STAT signaling (either through activating mutations, eg, *JAK2*V617F or *MPL*515L/K, within the signaling pathway or through mutations involving *CALR*) was demonstrated in PMF, post-PV MF, and post-ET MF [3-5], although these mutations have not been detected in MDS with BMF. Moreover, there is accumulating evidence for the role of deregulated proinflammatory cytokine expression and the impaired bone marrow microenvironment in BMF. The clinical phenotype in PMF, post-PV MF, and post-ET MF is a consequence of both the primary clonal myeloproliferation and a secondary inflammatory state by bone marrow stromal changes and an aberrant cytokine milieu [6]. A major biological hallmark of MF is a significant elevation in circulating proinflammatory cytokines. Transforming growth factor β (TGF-β) is a pleiotropic cytokine that potently stimulates fibroblasts to produce extracellular matrix proteins [7]. It also increases the expression of proteases that inhibit enzymes involved in the degradation of the extracellular matrix [7]. Experimental studies have demonstrated that TGF-β is important in the development of MF in animal models [8]. Elevated levels of TGF-β have also been documented in patients with BMF [9]. Therefore, anti–TGF-β therapy is a potentially promising strategy for treating patients with BMF [10].

Recently, it has been reported that mast cells are associated with fibrosis in renal interstitial fibrosis [11,12]. Fibrosis in IgG4-related diseases was believed to be caused by TGF-β–producing regulatory T cells [13]. However, current studies show that TGF-β– and interleukin (IL) 13–producing mast cells, and not regulatory T cells, are the primary participants in fibrosis [14,15]. We investigated the mechanism of BMF using the bone marrows from patients with PMF, post-ET MF, and MDS with BMF. This included histologic examination of mast cell infiltration and TGF-β and IL-13 production in bone marrows of patients with fibrosis compared with those of controls.

2. Materials and methods

2.1. Case samples

We analyzed 22 bone marrows from patients with confirmed fibrosis; of these, 8 patients had been diagnosed as having PMF, 5 had post-ET MF, and 9 had MDS with BMF. The average age of all patients was 71.3 years (range, 53-87 years). The average age of patients with PMF was 75.2 years (range, 63-87 years), that of patients with post-ET MF was 67.4 years (range, 61-81 years), and that of patients with MDS with BMF was 70.0 years (range, 53-78 years). Fifteen normal bone marrows were used as controls and were obtained from subjects who had been biopsied because of initial suspicions of involvement by lymphoma. The average age of control subjects was 69.3 years (range, 52-83 years). Ethical approval was obtained from the Ethics Committee of Osaka Medical College (No. 1724). Informed consent was obtained from all subjects. All experiments were performed in accordance with relevant guidelines and regulations.

2.2. Histopathology and immunohistochemical staining

Bone marrows obtained from each patient by biopsy were fixed in 10% buffered formalin, embedded in paraffin, cut into 4-μm-thick sections, and stained with hematoxylin and eosin. The extent of fibrosis was analyzed in bone marrows by silver impregnation stain and Masson trichrome stain. Grading of BMF was evaluated according to the European consensus on grading of BMF [1]. Immunohistochemical staining was performed in the paraffin sections using EnVision HRP (Dako, Glostrup, Denmark) for mouse monoclonal antihuman mast cell tryptase primary antibody (AA1; Dako) and using EnVision G/2 system/AP (Dako) for mouse monoclonal antihuman TGF-β antibody (TGFB17; Novocastra, Newcastle-upon-Tyne, UK) or rabbit polyclonal antihuman IL-13 primary antibody (Atlas Antibodies, Stockholm, Sweden).

We also performed dual immunofluorescence staining in the paraffin sections with the following antibody pairs: (1) mouse monoclonal antihuman TGF-β antibody (TGFB17; Novocastra) and rabbit monoclonal antihuman mast cell tryptase antibody (EPR8476; Abcam, Cambridge, MA), rabbit monoclonal antihuman CD34 antibody (EP373Y; Abcam), or rabbit polyclonal antihuman CD42b antibody (polyclonal; Protein Tech Group, Rosemont, IL) and (2) rabbit polyclonal antihuman IL-13 antibody (Atlas Antibodies) and mouse monoclonal antihuman mast cell tryptase antibody (AA1; Dako), mouse monoclonal antihuman CD34 antibody (QBEnd/10; Novocastra), or mouse monoclonal antihuman CD42b antibody (GP1b; Novocastra). Goat polyclonal antimouse IgG antibody (Alexa Fluor594; Abcam) and goat polyclonal antirabbit IgG antibody (Alexa Fluor488; Abcam) were used as chromogen-labeled secondary antibodies. Nuclei were stained with 40, 6-diamidino-2-phenylindole (Abcam). Images were captured by a fluorescence microscopy (BZ-X700; Keyence, Tokyo, Japan). Total bone marrow area, which excluded the region of bone trabeculae and marrow adipocytes, was measured by objective quantitative analysis using the WinROOF image processing software program (Mitani, Tokyo, Japan). The mast cells in the entire region of each bone marrow were counted using an ocular grid inserted into the eyepiece of a microscope by 2 pathologists, and counts were expressed as cells per square millimeter.

2.3. Statistical analysis

Results for continuous variables are shown as mean ± SE. The difference of frequency of infiltrating mast cells between bone marrows with fibrosis and control bone marrows was