

# Short Communication

## Mast Cells Are a Major Source of Basic Fibroblast Growth Factor in Chronic Inflammation and Cutaneous Hemangioma

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**Mast cells play an essential role during development of inflammation after chemical and immunological insults and have been implicated in tissue fibrosis and angiogenesis. The exact contribution of mast cells to these conditions is largely unknown. In this study, we found that a potent angiogenic and mitogenic polypeptide, basic fibroblast growth factor (bFGF), is localized to the majority of mast cells from normal skin and lung and in tissue samples characterized by fibrosis, hyperplasia, and neovascularization. Using specific antibodies to mast cell tryptase, tissue macrophage, and bFGF, we demonstrate that cytoplasmic bFGF immunoreactivity is localized to  $96.8 \pm 9.6\%$  of tryptase-positive cells in human fibrotic lung tissue ( $n = 10$ ),  $82.3 \pm 6.9\%$  of tryptase-positive cells in rheumatoid synovia ( $n = 6$ ), and  $93.1 \pm 4.8\%$  of tryptase-positive cells in skin hemangioma ( $n = 5$ ). Moreover, these tryptase-positive cells comprise a major portion (86 to 97%) of nonvascular cells exhibiting cytoplasmic bFGF staining in these tissues. In contrast, macrophage-like cells contribute less than 10% of the bFGF-positive cells in the same samples. The specificity of the immunostaining**

**results was supported by the finding that cultured human mast cells (HMC-1) express both bFGF mRNA and protein. Our data indicate that mast cells, a primary source of heparin, also serve as a significant source of a heparin-binding growth factor, bFGF, in these disease processes. These observations suggest that mast cells may contribute to these pathological conditions by releasing this polypeptide. (Am J Pathol 1995, 147:564-573)**

Increasing evidence suggests that mast cells (MCs) play an important role in inflammatory processes. For example, MC-deficient mice have reduced inflammation in ozone- and silica-induced mouse lung injury models.<sup>1,2</sup> Replenishing the MC-deficient animals with MCs restored the inflammation to its full extent. MCs are also required for full expression of cutaneous acute inflammation induced by phorbol ester<sup>3</sup> and antigen-induced arthritis in mice.<sup>4</sup> These studies indicate that MCs are important for tissue inflammatory responses to different types of injury. However, the roles of MCs in chronic inflammation remain largely unknown. Recent studies suggest that growth factors and cytokines, such as tumor necrosis factor- $\alpha$  and interleukins (ILs) are important inflammatory mediators related to MCs (for review, see Ref. 5). Bradding et al<sup>6</sup> recently immunolocalized IL-4, -5, and -6 to MCs in normal and inflamed nasal

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**Table 1.** Immunocolocalization of bFGF and Cell-Type Specific Antigens in Tissue Sections

Source	n	bFGF/Tryptase*	Tryptase/bFGF†	bFGF/HAM56‡	HAM56/bFGF§
Lung	10	96.8 ± 9.6%	95.7 ± 4.7%	<2%	<5%
Hemangioma	5	93.1 ± 4.8%	97.2 ± 3.6%	ND	ND
Synovium	6	82.3 ± 6.9%	86.2 ± 9.1%	<5%	<10%

Data (mean ± SD) are based on counting 150 to 500 cells per section. n, number of patients; ND, not done.

\*Percentage of tryptase-positive cells that coexpress bFGF.

†Percentage of bFGF-positive cells that are also stained for MC tryptase. bFGF-positive cells are the cells that exhibit cytoplasmic staining for bFGF.

‡Percentage of HAM56-labeled cells that are stained for bFGF. Endothelial cells were excluded.

§Percentage of bFGF-positive cells that are labeled by HAM56.

mucosa. However, the profile of MC-related growth factors *in vivo* is far from clear.

MC hyperplasia has been implicated in diverse fibrotic or proliferative diseases such as pulmonary fibrosis, scleroderma, neurofibromatosis, and psoriasis (for review, see Refs. 7–9). For example, an increased number of MCs are found in synovium from patients with rheumatoid arthritis,<sup>10,11</sup> in lung tissue from patients with idiopathic fibrosis,<sup>12</sup> and in skin from patients with scleroderma.<sup>13</sup> The exact contribution of MCs to these proliferative/fibrotic conditions is a matter of speculation. A variety of MC products are suspected to be disease mediators. They include histamine, tryptase, heparin, prostaglandin E, and platelet-activating factor. Their exact roles in MC-associated fibrotic/proliferative changes are a subject of investigation.

MCs also have been implicated in neovascularization. Increased numbers of MCs have been noted in diseases associated with neovascularization such as wound repair, rheumatoid arthritis,<sup>10,11</sup> diabetes mellitus,<sup>14</sup> hemangioma,<sup>15</sup> and other types of tumors (for a review, see Ref. 16). Kessler et al<sup>17</sup> reported that a marked (40-fold) increase in the number of MCs was found to precede angiogenesis induced by tumor implants on the chick chorioallantoic membrane. Recently, in a series of experiments, Norrby et al<sup>18–20</sup> demonstrated that active MC secretion induced by repeated intraperitoneal injection of compound 48/80, a highly selective MC secretagogue, resulted in marked mesenteric neovascularization in rats and mice as determined by vascularized area and the vascular density. More recently, Jakobsen<sup>21</sup> reported that the vascular changes in this experimental model included neovascularization and remodeling that lasted over a period of 2 months. Such marked organized angiogenic responses of long duration do not seem to be attributable to the structurally simple and short-lived mediators, such as histamine, released from MCs.

Basic fibroblast growth factor (bFGF) is a multifunctional polypeptide that affects growth and differentiation of a broad spectrum of cell types (for re-

views, see Refs. 22 and 23). It is a potent mitogenic and chemotactic factor and has been implicated in the wound healing process.<sup>24–26</sup> It stimulates fibroblast proliferation *in vitro* and promotes formation of granulation tissue during wound healing *in vivo*. Recent studies have directly linked this mitogenic factor to fibroproliferative disorders such as Dupuytren's contracture, pulmonary fibrosis, and carbon tetrachloride-induced hepatic fibrosis.<sup>27–29</sup> However, MCs were not considered to be a prime source of bFGF in these conditions. bFGF is also one of the most potent angiogenic factors known and has been shown to induce angiogenesis *in vitro* as well as *in vivo*. In the course of characterizing the distribution of bFGF in inflamed tissues, we noted that a group of tissue cells that showed cytoplasmic staining for bFGF also exhibited a distribution pattern similar to that of MCs. We therefore used specific antibodies to identify these cells. We elected to study tissue from three diseases that are notable for a recognized contribution by MCs and/or prominent neovascularization or fibrosis. Results from dual immunohistostaining of the tissues with anti-bFGF and cell-type-specific antibodies and from analysis of a cultured human MC line indicate that MCs may function as a major source of bFGF. Our study suggests that bFGF may contribute to cell proliferation and angiogenesis associated with MCs *in vivo*.

## Materials and Methods

### Immunohistochemistry

Human tissue samples were obtained from patients with idiopathic pulmonary fibrosis, rheumatoid arthritis, and cutaneous hemangioma (Table 1). Hemangioma specimens were selected on the basis of prominence of MC infiltration and dense vascularity. In addition, three normal skin samples and two lung samples from patients who died of accidental trauma were studied. The samples were fixed in neutralized buffered formalin and subsequently embedded in paraffin. A three-step avidin-biotin complex (ABC)

**Table 2.** List of Primary Antibodies Used in This Study

Antibody	Species	Dilution (concentration)	Treatment	Source
Anti-bFGF <sup>†</sup>	Mouse	1:20,000 (<3 µg/ml)	Hyaluronidase	ZymoGenetics, Inc., Seattle, WA
Anti-bFGF	Rabbit IgG	1:500 (2 µg/ml)	Hyaluronidase	Biomedical Technologies, Inc., Stoughton, MA
Anti-bFGF*	Rabbit IgG	1:500 (2 µg/ml)	(Immunoblot)	Santa Cruz Biotech, Santa Cruz, CA
Anti-tryptase	Mouse IgG	1:200 (0.5 µg/ml)	Hyaluronidase	Dako Corp., Carpinteria, CA
Anti-CD68 (KP1)	Mouse IgG	1:100 (3.7 µg/ml)	Hyaluronidase	Dako Corp.
HAM56	Mouse IgM	1:500 (1.4 µg/ml)		Dako Corp.

\*This antibody is raised against full length human recombinant bFGF and does not cross-react with acidic FGF upon immunoblot analysis.<sup>56</sup> Clone number of this antibody is 148.6.1.1.1. The listed dilution is for a two-step indirect method. For the ABC method, the dilution is 1:10<sup>6</sup> (<0.1 µg/ml).

<sup>†</sup>Antibodies also used for immunoblot analysis.

(Vector Laboratories, Burlingame, CA) method or alkaline phosphatase-anti-alkaline-phosphatase (APAAP) method (Dako Corp., Carpinteria, CA) was used to localize bFGF and other cell markers as described elsewhere.<sup>30</sup> Information about the antibodies used in this study is summarized in Table 2. The antibody-antigen complexes were visualized by incubation for 30 minutes in fast red substrate (BioGenex Laboratories, San Ramon, CA) or Vector blue (Vector) containing 2 mmol/L levamisole according to the suppliers' instructions. The sections were counterstained with Gill III hematoxylin when indicated and mounted in crystal mount (Biomedica Corp., Foster City, CA).

For double immunostaining of bFGF and tryptase or macrophages, the sections were first labeled for bFGF by the ABC-AP method with Vector blue as substrate. Potential nonspecific binding resulting from the first labeling was blocked with the biotin/avidin system following the vendor's instruction (Vector). The sections were then incubated with anti-tryptase at 1:200 (0.5 µg/ml) or HAM56 at 1:50 (2.5 µg/ml) overnight at 4°C followed by incubation with biotinylated horse anti-mouse at 1:200 and then avidin-fluorescein isothiocyanate at 1:100 for 1 hour at room temperature. Brief washes were performed after each step.

For CD68/tryptase double labeling, the samples were first labeled by antibodies to macrophages by the ABC-AP method with fast red as the substrate. The second labeling was performed by the APAAP method with Vector blue as substrate.

Two control groups were used to confirm the staining specificity: (1) replacing the specific primary antibodies with nonimmune immunoglobulins of the same isotype at the same concentration from the same species as the antibodies and (2) washing the sections with phosphate-buffered saline containing 2 mol/L NaCl after enzymatic treatment. Nonimmune immunoglobulin was also used to replace the primary antibody during the second labeling to determine the specificity of the second labeling.

At least two specimens from each case were examined for each antibody. Two approaches were used to determine the spatial relationship of cells labeled with different antibodies or with toluidine blue. In the first, the tissue sections were cut sequentially at 2 to 3 µm and stained separately with antibodies to bFGF, macrophages, and tryptase or toluidine blue. In the second, the sections were double stained for bFGF/tryptase, bFGF/HAM56, or tryptase/CD68 by combined colorimetric and fluorescent methods. The spatial relationship of positive cells labeled by two different antibodies on each section was determined by bright field and fluorescent microscopic examination. The samples were examined by at least two independent researchers. A total of 500 cells from three randomly selected areas in samples from fibrotic lung and cutaneous hemangioma were examined. In samples from rheumatoid synovium, cells (ranging from 150 to 300) were counted in four randomly selected areas which included lining synovium and underlying connective tissue regions.

### Cell Culture

The human MC line, HMC-1, was grown in Iscove's medium with 10% defined bovine calf serum (HyClone, Logan, UT). Cells were plated at 1 × 10<sup>5</sup> cells/ml and maintained in a water-vapor-saturated atmosphere with 5% CO<sub>2</sub> for 2 days. Cells reaching 4 × 10<sup>5</sup> cells/ml were used for the study.

To induce bFGF expression by HMC-1 cells, the cells were plated at 1.5 × 10<sup>5</sup> cells/ml and maintained for 24 hours. Then, phorbol 12-myristate 13-acetate (TPA) and calcium ionophore A23187 dissolved in dimethyl sulfoxide (Sigma Chemical Co., St. Louis, MO) were added to 80 nmol/L and 1 µmol/L, respectively. Cells were harvested 24 hours after stimulation and analyzed by immunoblot and reverse transcription-polymerase chain reaction (RT-PCR) (see below).

### *Immunoblot Analysis of Cell Lysates for bFGF*

Approximately  $10^7$  HMC-1 cells were lysed in 1 ml of lysis buffer containing 10 mmol/L HEPES (pH 7.3), 0.4 mol/L NaCl, 1 mmol/L  $MgCl_2$ , 5% Nonidet P-40, 10 mmol/L EDTA, 2 mmol/L phenylmethylsulfonyl fluoride, 2  $\mu g/ml$  leupeptin, and 2  $\mu g/ml$  aprotinin. A 100- $\mu l$  aliquot of each lysate was incubated overnight at 4°C with 25  $\mu l$  of precleaned heparin acrylic beads (Sigma Chemical Co.). The heparin beads were collected by centrifugation, washed three times with the lysis buffer containing 0.8 mol/L NaCl, equilibrated with 10 mmol/L HEPES at pH 7.4, and then subjected to gel electrophoresis and immunoblot analysis as described elsewhere.<sup>31</sup> Two different anti-bFGF antibodies were used for this assay to assure specificity: a monoclonal anti-bFGF at 1:10,000 and a polyclonal rabbit anti-bFGF at 1:500 (Santa Cruz Biotechnology, Santa Cruz, CA). Human recombinant bFGF (145-amino-acid form) (R&D Systems, Minneapolis, MN) was used as a positive control.

### *Detection of bFGF mRNA by RT-PCR*

A total of 150 ng of the RNA from stimulated HMC-1 cells and a rheumatoid synovium sample was reverse transcribed as previously described.<sup>32</sup> For PCR, 4  $\mu l$  of the reverse-transcribed material from these samples were amplified in 50- $\mu l$  reaction volumes.<sup>32</sup> Two different sets of primers were used. They contain the sequences unique to bFGF that are not found in other known genes. The sequence of the first set (sense 5'-TGT ACT GCA AAA ACG GGG GCT TCT CCT GCG CAT CC-3' and antisense 5'-CGT AAC ACA TTT AGA AGC CAG TAA TCT TCC ATC TTC C-3') corresponded to the region of nucleotides 561 to 760 of a human bFGF mRNA. The sequence of the second set (sense 5'-CAA GCA GAA GAG AGA GGA GTT GTG TC-3' and antisense 5'-CAG TTC GTT TCA GTG CCA CAT ACC-3') included the region from 659 to 857 of human bFGF mRNA. A restriction site for endonuclease *HinfI* is located at position 789. Treatment of the PCR product generated by the second pair of primers should result in fragments of 130 and 68 bp.

For the endonuclease digestion assay, PCR-amplified products were treated with *HinfI* (GIBCO BRL, Grand Island, NY) for 1 hour at 37°C. The reaction mixture (30  $\mu l$ ) contained 50 mmol/L Tris (pH 8.0), 10 mmol/L  $MgCl_2$ , 50 mmol/L NaCl, and 10 U of *HinfI*. The digestion fragments were analyzed by gel electrophoresis as described above.

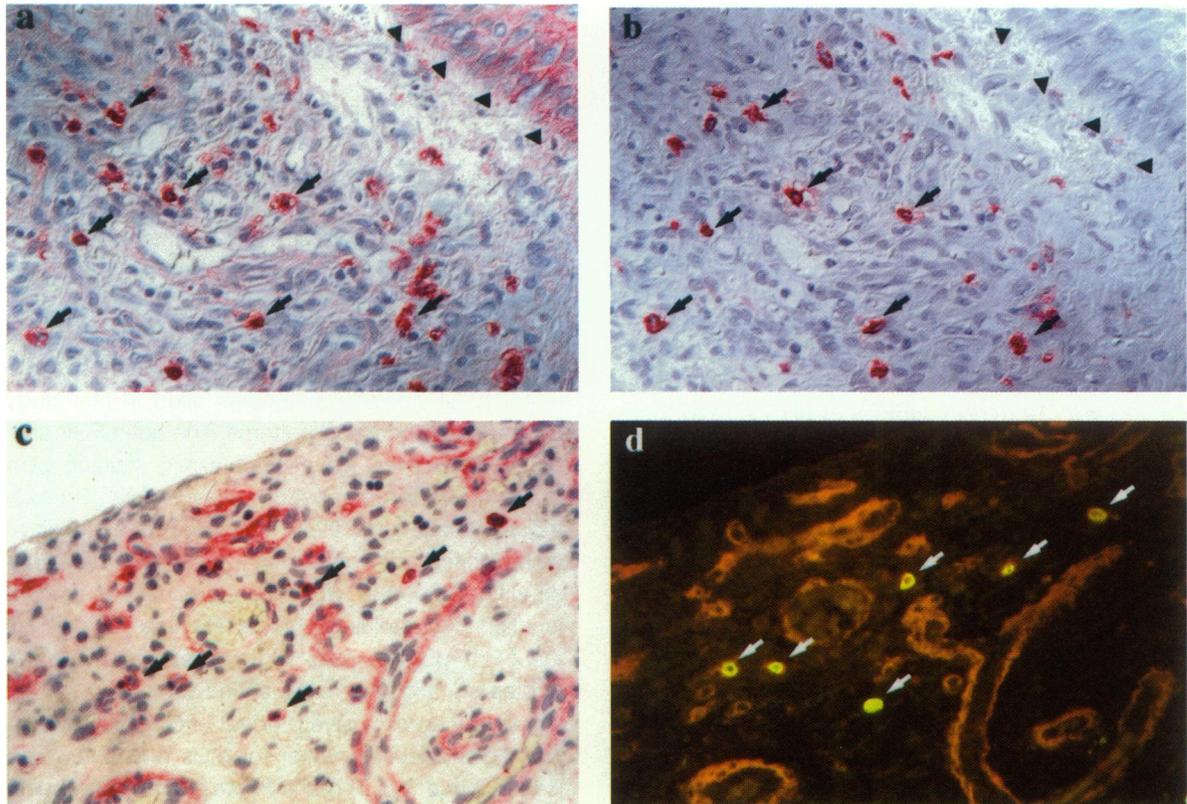
## **Results**

### *Immunohistochemistry*

When the tissue samples were labeled with antibodies specific to bFGF, staining for bFGF was associated with tunica media and tunica adventitia of small veins and venules, hyperplastic synoviocytes, and epidermal cells (Figure 1, a and c). Intense vascular staining for bFGF was found in all rheumatoid synovia examined. The intensity of bFGF staining associated with vascular cells exhibited great variability in fibrotic lung and cutaneous hemangioma. Two different bFGF-specific antibodies used in this study gave identical staining patterns. The anti-bFGF antibodies selectively gave cytoplasmic staining of a group of scattered connective tissue cells. The distribution pattern of these scattered bFGF-positive cells closely resembled that of MCs. Cell-type-specific antibodies were therefore utilized in conjunction with toluidine blue staining to identify the bFGF-positive cells in these samples. Human tryptase is a glycosylated serine proteinase that is essentially an exclusive product of MCs and is considered a specific marker for MCs (for review see Refs. 33 and 34). The anti-tryptase antibody specifically reacts with tissue MCs by immunohistochemistry.<sup>35-37</sup> As macrophages are commonly accepted as a major source for tissue cytokines/growth factors and are thought to play a major role during angiogenesis by releasing angiogenic factors including bFGF,<sup>38</sup> tissue samples were also stained with two monoclonal antibodies (KP1 and HAM56) to human monocyte/macrophage.<sup>39,40</sup>

Careful examination of 2- to 3- $\mu m$  sequential sections labeled by anti-bFGF and anti-tryptase separately revealed almost identical distribution patterns of bFGF- and tryptase-positive cells (Figures 1, a and b, and 2, a and b). Similar results were obtained by staining of the sequential sections with toluidine blue and anti-bFGF (Figure 2, c and d). The anti-tryptase did not label endothelial cells, smooth muscle cells, lymphocytes, macrophages, basement membrane, or epidermal cells in any specimen examined. In contrast, the distribution pattern of cells labeled by anti-macrophages exhibited no resemblance to that of bFGF-positive cells or tryptase-positive cells (Figure 2, a and b).

In normal skin samples, staining for bFGF was also associated with epidermal and vascular cells (data not shown). Cytoplasm staining for bFGF was also found in scattered connective tissue cells that were labeled by anti-tryptase on the adjacent sequential section. Similar results were also ob-



**Figure 1.** Immunohistological localization of bFGF and MCs in hemangioma and rheumatoid synovium. **a** and **b**: Paired 2- $\mu$ m sequential sections from hemangioma stained for bFGF (**a**) and tryptase (**b**). The sections were counterstained with hematoxylin. Arrows indicate some of the cells that react with both anti-tryptase and anti-bFGF antibodies. Note that only anti-bFGF labels epidermis (arrowheads). Panels **c** (brightfield) and **d** (fluorescence) are from a section from rheumatoid synovium double labeled with anti-bFGF (pink) and anti-tryptase (fluorescent green). Note the colocalization of bFGF with the MC marker. Original magnification,  $\times 400$ .

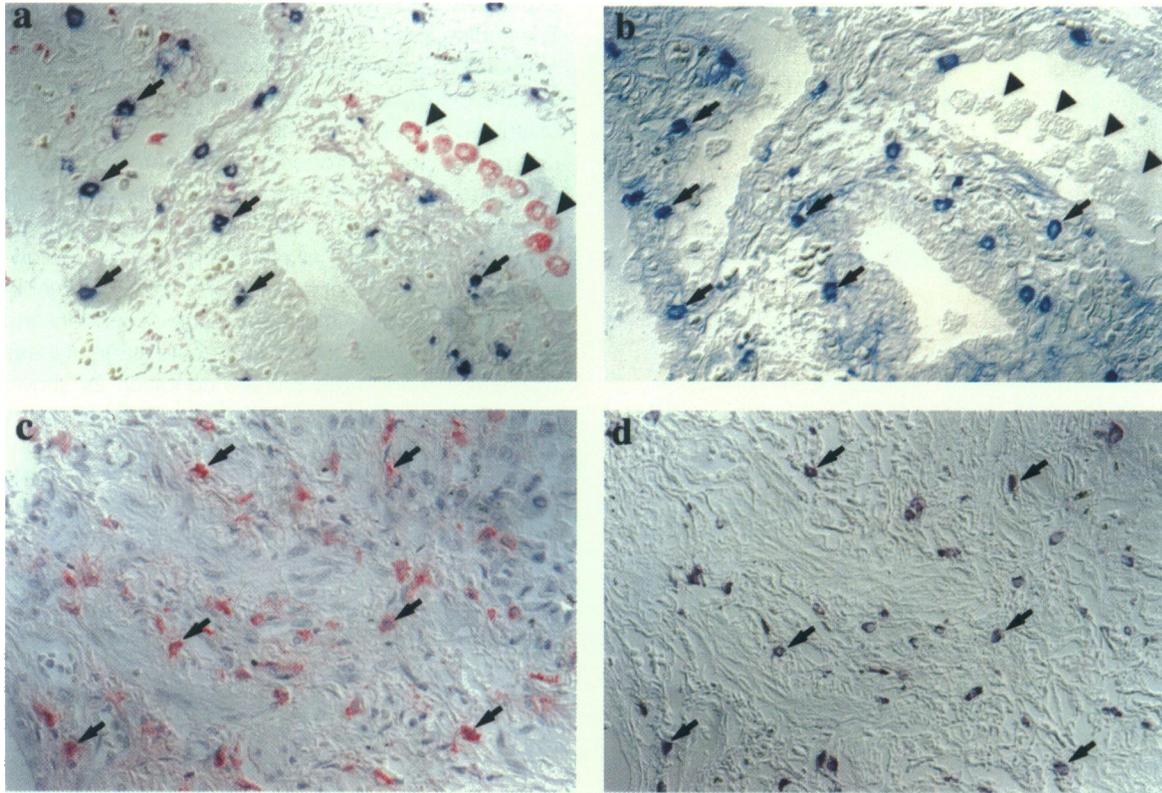
served in the normal lung sample. However, in both normal skin and lung samples, only a limited number of these bFGF- and tryptase-positive cells were present. Nevertheless, the majority (>70%) of the tryptase-positive cells exhibited staining for bFGF. This observation was also confirmed by double immunohistochemical staining (see below).

#### Double Immunohistochemical Staining

To confirm the association of bFGF and MCs, double immunohistochemical staining was then performed. Double immunohistochemical/immunofluorescent staining for bFGF/tryptase revealed that the majority of tryptase-positive cells were also labeled by anti-bFGF in all samples examined (Figure 1, c and d). More importantly, these MCs comprise a large fraction of cells with cytoplasmic staining for bFGF in these samples (Table 1). In contrast, the majority of cells labeled by the antibodies to macrophages were devoid of cytoplasmic bFGF staining (Figure 2b) and

the fraction of bFGF-positive cells reactive to macrophage antibodies was very small (Table 1). Anti-tryptase and anti-macrophage antibodies labeled two different cell populations with negligible overlap in all samples examined. In the specimens of fibrotic lung, for example, tryptase-positive cells were mainly localized in the interstitial tissue and fibrotic regions, whereas the majority of cells within alveoli were labeled with antibodies to macrophages (Figure 2a). The staining pattern of each antibody by double labeling was consistent with that obtained by single labeling. No nonspecific staining caused by cross-reaction between antibodies and labeling reagents for the first and second labeling was observed after protocols described here (data not shown).

Because tissue MCs have been reported to exhibit nonspecific staining as a result of their intrinsic affinity to avidin<sup>41,42</sup> and to some types of immunoglobulins,<sup>43</sup> several approaches were carried out to confirm the specificity of tryptase and bFGF staining. In addition to a standard control, an alternative method, APAAP staining, was used to label the specimens



**Figure 2.** Staining of fibrotic lung tissue for bFGF, MCs, and macrophages. **a** and **b**: Sequential sections stained for CD68 (pink) and tryptase (dark blue) (**a**) and for bFGF (dark blue) (**b**). Arrows indicate some cells labeled by both anti-tryptase and anti-bFGF. Note the absence of bFGF staining in CD68-positive cells (arrowheads). **c** and **d**: Also paired sequential sections stained with anti-bFGF (**c**) and toluidine blue (**d**). Arrows indicate some of the cells labeled by both anti-bFGF and toluidine blue. Original magnification,  $\times 400$  (Nomarski).

and the result obtained was compared with that obtained by the ABC method. Moreover, a brief wash of tissue sections with a buffer containing 2 mol/L NaCl before staining, a procedure known to abolish bFGF staining presumably as a result of dissociation of bFGF from heparin,<sup>31</sup> was also performed. As we expected, replacing specific primary antibodies with a counterpart immunoglobulin from nonimmune animals completely abolished the staining. The APAAP method gave a staining identical to that obtained by the ABC method for each antibody used (data not shown). Washing with buffer containing 2 mol/L NaCl selectively abolished bFGF staining but did not affect the tryptase staining (data not shown). These results indicated that staining of the MCs by both anti-tryptase and anti-bFGF was not a result of the intrinsic affinity of heparin to avidin or immunoglobulins.

#### *bFGF-Like Polypeptide and mRNA Are Detectable in a Human Mast Cell Line*

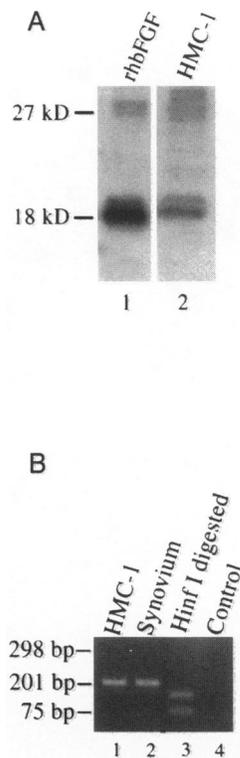
To examine the association of bFGF and MCs further, immunoblot analysis was used to detect bFGF-

like molecules produced by MCs *in vitro*. A human MC line, HMC-1,<sup>44</sup> was studied. Immunoblot analysis of total protein from HMC-1 cells stimulated with TPA and A23187 revealed a dominant band with an apparent molecular mass of 17 kD that exhibited migration behavior similar to that of human recombinant bFGF (Figure 3).

Amplification of mRNA from HMC-1 treated with TPA/A23187 by RT-PCR with two different pairs of primers gave rise to single bands with the predicted mobility (approximately 200 bp) upon gel electrophoresis (Figure 3). The specificity of the RT-PCR was confirmed by endonuclease mapping. Treatment of the amplified products with restriction endonuclease, *Hinf*I, generated a banding pattern as predicted according to the sequence of bFGF to be amplified (Figure 3).

#### **Discussion**

In this report, we present evidence for the first time indicating that, in addition to vascular cells, MCs may serve as a significant source of tissue bFGF in



**Figure 3. A:** Immunoblot analysis of stimulated HMC-1 cell lysates. Lane 1 contains recombinant human bFGF. Lane 2 contains lysate from TPA/A23187-stimulated HMC-1 cells. A predominant band with an apparent molecular mass of 17 kD that exhibits identical migration as human recombinant bFGF is detected by anti-bFGF. **B:** Detection of bFGF mRNA from HMC-1 cells and rheumatoid synovium by RT-PCR. Samples from TPA/A23187-treated HMC-1 cells (lane 1) and rheumatoid synovium (lane 2) give rise to a single band of ~200 bp as predicted according to the sequence of human bFGF mRNA. Treatment of the amplified product with HinfI generates bands of 130 bp and 68 bp as predicted (lane 3). mRNA quantitation and integrity were verified for all samples by RT-PCR with primers for a constitutive gene, cyclophilin<sup>32</sup> (data not shown here). As a negative control (lane 4), RNA was omitted from the RT reaction.

angiogenic and some chronic inflammatory conditions. Using specific antibodies, we demonstrate a marked spatial correlation between bFGF immunoreactivity and MCs in all samples examined by immunohistochemistry. Double immunohistochemical staining revealed that the majority of tryptase-positive cells in fibrotic lung samples and cutaneous hemangiomas were also stained for bFGF and *vice versa*, indicating that most of the MCs were closely associated with bFGF. Although the fraction of tryptase-positive cells reactive with anti-bFGF in rheumatoid synovia is somewhat lower than that in fibrotic lung tissue and hemangioma, it still constitutes a major portion of the nonvascular bFGF-positive cells in these samples (see Table 1). Such marked correlation indicates that MCs may function as a source of tissue bFGF during inflammation and neovascularization. Consistent with this hypothesis is the finding that a stimulated, cultured human MC line

expresses bFGF at both protein and mRNA levels. The finding that bFGF immunoreactivity was found in a much larger number and fraction of MCs than that of monocytes/macrophages in all tissue specimens examined strongly argues against the hypothesis that monocytes/macrophages are the major source of tissue bFGF during inflammation in lung<sup>45-47</sup> and neovascularization.<sup>38</sup> Preliminary data from a recent study by Inoue et al<sup>48</sup> also support this notion. However, it is possible that expression of bFGF by monocytes/macrophages is disease stage dependent and is more prominent in the early or acute stage of these diseases<sup>28</sup> than in the chronic condition as shown here. Studies in progress on animal models may clarify this.

Immunohistochemically detected bFGF in MCs may come from two potential sources. It may be synthesized by MCs and/or it may be synthesized by other cells and stored in MCs. As it has been shown that bFGF has high affinity to heparin and that connective tissue MCs contain abundant heparin, it is conceivable that MCs may function only as a storage compartment of tissue bFGF. However, our *in vitro* study clearly demonstrated that cultured human MCs do express bFGF at both mRNA and protein levels and established that at least a subpopulation of MCs has the potential to express bFGF. It is reasonable to predict that MCs may not only function as a storage site for bFGF but also produce bFGF *in vivo* when activated. Preliminary data from a study by Reed et al<sup>49</sup> using *in situ* hybridization also indicated that bFGF is synthesized by MCs *in vivo*.

MCs are highly heterogeneous in terms of their morphology, function, and byproducts such as heparin, tryptase, or chymase (for review, see Refs. 7 and 50). For example, expression of chymase by human MCs is associated with cell development and differentiation. Functional heterogeneity has been found to exist in MCs isolated from the same tissue.<sup>51</sup> The finding that bFGF is expressed by MCs in several different organs suggests that this growth factor may be associated with some fundamental functions of MCs. In support of this is our recent finding that several cultured murine MC lines express a substantial amount of bFGF as judged by Northern and Western blot analyses and enzyme-linked immunosorbent assay (unpublished observation).

Although bFGF can be detected in MCs in normal skin and lung tissues, increased numbers of MCs in diseased conditions may result in increased levels of local bFGF that may be responsible for a wide spectrum of MC-associated pathological changes. As bFGF is a potent mitogenic and angiogenic polypeptide, our findings may help to explain the close as-

sociation of MCs with chronic inflammation and angiogenesis. First, MCs have been implicated in neovascularization, whereas bFGF is one of the most potent angiogenic factors known. Thus, neovascularization induced by MC degranulation<sup>18-20</sup> is likely to be mediated by bFGF. Second, increased numbers of MCs have been found in tissues with chronic inflammation and fibrosis. Proliferation of fibroblasts, as is characteristic of rheumatoid synovium, is associated with an increase in MCs and may be causally related to bFGF released from these cells.

In contrast to most other growth factors that function through a paracrine mechanism, bFGF lacks a signal peptide sequence for secretion. Despite intensive studies, the mechanism(s) of bFGF release is still a subject of controversy. bFGF exhibits high affinity for heparin and formation of a bFGF-heparin complex is known to stabilize bFGF structurally and preserve its bioactivity by protecting it from enzymatic degradation.<sup>52-54</sup> Moreover, heparin and heparan sulfate are required for binding of bFGF to its receptors and, therefore, for induction of cellular responses to bFGF.<sup>55</sup> The MCs' ability to express bFGF, together with their two other unique characteristics, high heparin content and degranulation, renders them an ideal candidate for regulation of local bFGF levels and bioactivity. First, increased bFGF expression may result from the tissue response to a specific insult(s). Second, the presence of abundant heparan sulfate and heparin with high binding affinity to bFGF implies a large capacity for bFGF storage. In addition, MCs may potentiate the bioactivity of bFGF through release of heparin. Third, MC degranulation may provide an efficient mechanism for bFGF release and another line of regulation of tissue bFGF levels. Preliminary data from our ongoing study of several murine MC lines also show that cultured MCs synthesize a substantial amount of bFGF, and bFGF appears to be associated with cytoplasmic granules by ultrastructural immunolocalization (unpublished observations). These observations strongly implicate MC degranulation as a potential mechanism of bFGF release.

In summary, our data show that MCs are a major source of bFGF. In contrast to MCs, macrophages rarely expressed bFGF detectable by immunohistochemical staining in the samples examined. Our findings suggest that bFGF may contribute to cell proliferation and angiogenesis associated with MC degranulation *in vivo*. The localization of bFGF to a cell that synthesizes heparin may help elucidate the physiology of bFGF. This study helps clarify the contribution of the MCs in angiogenesis, pulmonary fibrosis, and inflammatory joint disease.

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