Mast Cell Involvement in Fibrodysplasia Ossificans Progressiva

FRANCIS H. GANNON, MD, DAVID GLASER, MD, ROBERT CARON, MS, LESTER D.R. THOMPSON, MD, EILEEN M. SHORE, PhD, AND FREDERICK S. KAPLAN, MD

Fibrodysplasia ossificans progressiva (FOP) is a catastrophic genetic disorder of progressive heterotopic ossification associated with dysregulated production of bone morphogenetic protein 4 (BMP4), a potent osteogenic morphogen. Postnatal heterotopic ossification in FOP is often heralded by hectic episodes of severe post-traumatic connective tissue swelling and intramuscular edema, followed by an intense and highly angiogenic fibroproliferative mass. The abrupt appearance, intense size, and rapid intrafascial spread of the edematous preosseous fibroproliferative lesions implicate a dysregulated wound response mechanism and suggest that cells and mediators involved in inflammation and tissue repair may be conscripted in the growth and progression of FOP lesions. The central and coordinate role of inflammatory mast cells and their mediators in tissue edema, wound repair, fibrogenesis, angiogenesis, and tumor invasion prompted us to investigate the potential involvement of mast cells in the pathology of FOP lesions. We show that inflammatory mast cells are present at every stage of the development of FOP lesions and are most pronounced at the highly vascular fibroproliferative stage. Mast cell density at the periphery of FOP lesions is 40- to 150-fold greater than in normal control skeletal muscle or in uninvolved skeletal muscle from FOP patients and 10- to 40-fold greater than in any other inflammatory myopathy examined. These findings document mobilization and activation of inflammatory mast cells in the pathology of FOP lesions and provide a novel and previously unrecognized target for pharmacologic intervention in this extremely disabling disease. Hum Pathol 32:842-848. This is a US government work. There are no restrictions on its use.

Key words: mast cells, fibrodysplasia ossificans progressiva, heterotopic ossification, osteogenesis.

Abbreviations: FOP, fibrodysplasia ossificans progressiva; BMP4, bone morphogenetic protein 4; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; VPF, vascular permeability factor; TGF, transforming growth factor.

Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disorder characterized by congenital malformation of the great toes and by progressive heterotopic ossification in defined anatomic patterns.1,2 Spontaneous and post-traumatic flare-ups of FOP are heralded by episodes of intense connective tissue edema with early histologic evidence of perivascular lymphocytic infiltration into skeletal muscle.3,4 Highly angiogenic fibroproliferative lesions appear within hours, spread rapidly along muscle planes, and evolve through an endochondral ossification process to form mature lamellar bone.5,6 Heterotopic ossification in FOP leads to immobilization of joints in the axial and appendicular skeleton, rendering movement impossible.2,9,10 Death frequently results from starvation due to ankylosis of the jaw or from complications of severe restrictive disease of the chest wall.2,9,11,12 Presently, there is no effective prevention or treatment.13

Although the genetic basis and pathophysiology of FOP are unknown, recent studies show overproduction of bone morphogenetic protein 4 (BMP4) in lesional lymphocytes, lesional proliferating fibroblasts, and lymphoblastoid cell lines from patients with FOP.1,3,4,7 Additionally, elevated levels of urinary basic fibroblast growth factor (bFGF), a potent angiogenic peptide, are noted during FOP flare-ups but not during disease quiescence.14 However, bFGF is not overproduced in lesional proliferating fibroblasts, implicating other cells as the source of bFGF. The intense soft tissue swelling and rapidly progressive muscle edema, fibroproliferation, and angiogenesis characteristic of early preosseous FOP lesions suggest involvement of an armada of inflammatory mediators at the leading edge of the lesion and points to a potential role for inflammatory mast cells in the spread of the disease process.

Mast cells mediate inflammatory reactions that are widely distributed in connective tissues. Mast cells arise from CD34+ pluripotent stem cells of hematopoietic origin, circulate through the blood as committed but undifferentiated cells, and migrate into numerous tissues including skeletal muscle, where they mature in the presence of c-kit-ligand (stem cell factor).15,16 Mast cells are indigenous to connective tissues and are found in close proximity to blood vessels and nerves.16 In normal skeletal muscle, mast cells are found sparsely distributed in the connective tissue interstitium that
MAST CELL INVOLVEMENT IN FOP (Gannon et al)

MATERIALS AND METHODS

Patient Population

Between 1988 and 1999, 174 patients with classic features of FOP were referred to the FOP clinic at the University of Pennsylvania School of Medicine and were seen at the Children’s Hospital of Philadelphia, at the Hospital of the University of Pennsylvania, or at one of the satellite clinics conducted by 2 of us (F.S.K. and D.L.G.). A definitive diagnosis of FOP was confirmed in all 174 patients on the basis of congenital malformation of the great toes and progressive heterotopic ossification in characteristic anatomic patterns. FOP protocols were approved by the institutional review boards of the Children’s Hospital of Philadelphia and the University of Pennsylvania, and informed consent was obtained from all patients or their parents.

FOP Biopsy Specimen Retrieval

All FOP lesional tissue was retrieved from the tissue repository at the Center For Research in FOP and Related Disorders at the University of Pennsylvania. Tissue blocks were originally procured after patient or parental permission was obtained. All lesional samples contained in the database were used. At the time of the study, paraffin-embedded tissue blocks of FOP lesions were available from 7 of the 174 classically affected FOP patients. Multiple blocks of the lesions were available for 3 of the 7 patients (4 girls and 3 boys), who ranged in age from 1 to 10 years at the time of the biopsies. Samples were obtained from fibrous tissue in deep fascia and from paravertebral, infraspinatus, frontalis, sternocleidomastoïd, vastus lateralis, and intercostal muscles. All specimens had been resected by means of an open biopsy of lesional tissue. The FOP lesional biopsies had been performed at other medical centers to exclude the diagnosis of a soft tissue neoplasm, and in none of the cases was the correct diagnosis suspected before surgery. FOP nonlesional muscle tissue was obtained from 3 children with FOP who underwent emergency surgical procedures for intercurrent problems.

Inflammatory Muscle Conditions

Specimens from normal control muscle tissue and genetic and nonparasitic inflammatory myopathies were retrieved from the files of the Armed Forces Institute of Pathology during the period of 1996 through 1998. One hundred six cases were included in the analysis and involved a variety of muscle groups (quadriceps, deltoid, mandible, omohyoid, biceps, trapezius, sternocleidomastoïd, gastrocnemius, sartorius, rectus, vastus lateralis, tibialis anterior, and diaphragm) and the following conditions: minimal inflammation, severe inflammatory myopathy, dermatomyositis, acute infection, scar, tumor invading into muscle, foreign body, infarct, and pan necrosis. A total of 62 men and 44 women ranging in age from 1 to 78 years were represented in this series.

Tissue Specimens and Histopathologic Evaluations

All tissue specimens had been fixed in neutral buffered formalin and embedded in paraffin. Specimens were sectioned at a thickness of 5 μm and stained with hematoxylin-eosin staining and have not been previously recognized or described in FOP pathology.

Little is known about the resident mast cell population in skeletal muscle. We have undertaken a comprehensive analysis of mast cell distribution in normal skeletal muscle, in uninvolved skeletal muscle from patients with FOP, in FOP lesions, and in inflammatory myopathies to determine if mast cells may be involved in the pathology of FOP lesions.

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<th>TABLE 1. Mast Cell Density in Normal Skeletal Muscle, Inflammatory Myopathies, and Fibrodysplasia Ossificans Progressiva Lesions</th>
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Abbreviation: HPF, high-power field.
FIGURE 1. Mast cell mobilization in FOP lesions: (panels 1a, 1b, and 1c) mast cells in early stages of FOP lesion formation; (panels 2a, 2b, and 2c) mast cells in later stages of FOP lesion formation. (Panel 1a) High-power photomicrograph of the earliest lesion of FOP (stage 1A). Lymphocytes surround a vessel (V) with a mast cell (arrow) in the surrounding connective tissue. The arrow points to the mast cell shown in the inset. (Chloracetate esterase staining; original magnification ×480; inset, original magnification ×1,600.) (Panel 1b) High-power field of a stage 1B FOP lesion. This next phase of progression reveals myocytes (M) with admixed myonecrosis and an adjacent mast cell (arrow). A vessel (V) is also noted. The arrow points to the mast cell shown in inset. (Chloracetate esterase staining; original magnification ×480; inset, original magnification ×1,600.) (Panel 1c) High-power photomicrograph of a stage 1C FOP lesion. This stage exhibits an early fibroproliferative lesion with abundant mast cells (arrow and arrowhead). The arrowhead points to the mast cell shown in the inset. (Chloracetate esterase staining; original magnification ×480; inset, original magnification ×1,600.) (Panel 2a) A stage 2A fibroproliferative lesion with vessels (V), fibroconnective tissue, and mast cells. M, myocytes. (Chloracetate esterase staining; original magnification ×480; inset, original magnification ×1,600.) (Panel 2b) A high-power field of a stage 2B fibroproliferative lesion showing cartilage (C) production, vessels (V) and a mast cell (arrow) adjacent to a vessel. The arrow points to the mast cell shown in the inset. (Chloracetate esterase staining; original magnification ×480; inset, original magnification ×1,600.) (Panel 2c) A high-power photomicrograph of a mature FOP lesion (stage 2C) shows bone (B) and cartilage (C) and a nearby mast cell (arrow). The arrow points to the mast cell shown in the inset. (Chloracetate esterase staining; original magnification ×480; inset, original magnification ×1,600.)

FIGURE 2. Hypothetical schema of pathophysiology of FOP lesions. Although mast cell involvement is unlikely to be responsible for formation of fibroproliferative lesions, mast cells may potentiate the severity of the inflammatory response and thus progressive spread of a lesion along tissue planes. FOP patients are genetically susceptible to BMP4-mediated heterotopic ossification.
MAST CELL INVOLVEMENT IN FOP (Gannon et al)

Tissue Injury

Lymphocyte recruitment to skeletal muscle

Mast cell chemotaxis and Mast cell proliferation (c-Kit receptor)

Mast cell degranulation

BMP4

TGF-β

Histamine heparin, & inflammatory mediators

bFGF; VEGF/VPF

Fibrosis
Inflammatory edema (early pre-osseous FOP lesion)

Stem Cell Factor (c-Kit ligand)

BMP4

Endochondral Ossification
groups of 10 high-power fields were counted in the muscle controls, and between 5 and 10 groups of 10 high-power fields were counted for the FOP tissue depending on the size of the biopsy specimen. Each of the pathologists (F.H.G. and L.D.R.T.) performed these counts individually, and the results were averaged. The starting point for the analysis was randomly chosen, but all fields were contiguous and within the lesional tissue. The counting was repeated on separate occasions to determine intraobserver and interobserver variation. Mast cells were identified as indigenous connective tissue cells with large central nuclei and large abundant metachromatic cytoplasmic granules when stained by cationic dyes, such as toluidine blue or Giemsa (because of the presence of sulfated glycosaminoglycans) or when stained by chloracetate esterase (because of the presence of chymotrypsin-like serine esterase activity). Intraobserver variation was <1%, and interobserver variation was 3%.

RESULTS

Mast Cells in Normal Muscle

Representative muscle groups were examined from axial, appendicular, cranial, truncal, caudal, dorsal, ventral, proximal, and distal regions of the body (Table 1). There was a remarkably consistent distribution and low density of mast cells in normal skeletal muscle from all anatomic sites and across all age ranges. There were no differences in the pattern or density of mast cell distribution between uninvolved skeletal muscle from normal controls and uninvolved skeletal muscle from patients with FOP (Table 1). Mast cells were limited in distribution to the connective tissue interstitium of normal skeletal muscle and uninvolved FOP muscle at a density of ≤0.1 mast cells per 10 high-power fields (Table 1).

Mast Cells in Inflammatory Myopathies

Mast cell density in inflammatory myopathies ranged from 0.1 to 0.5 mast cells per 10 high-power fields, regardless of the underlying etiology of the myopathic process (Table 1). Similar to normal skeletal muscle, the mast cells in inflammatory myopathies were limited to the interstitium in the majority of cases, with an occasional mast cell noted within the surrounding muscle.

Mast Cells in FOP Lesions

FOP lesions were staged histologically based on the pathologic and morphologic characteristics and have been designated 1A, 1B, 1C, 2A, 2B, and 2C as previously described. The 1A lesion (Fig 1, panel 1a) is characterized by an intense perivascular B-cell and T-cell lymphocytic aggregation before invasion into the surrounding muscle tissue. Even at this early stage, mast cells are associated with perivascular lymphocytic infiltrates and are 10-fold more abundant (1.0 mast cells per 10 high-power fields) than in normal control skeletal muscle. In stage 1B lesions (Fig 1, panel 1b), T lymphocytes have migrated from the perivascular space into the surrounding muscle. At this stage, mast cells are mobilized from the perivascular connective tissue interstitium of the dying skeletal muscle and are at a significantly higher concentration than in normal control muscle tissue at any site in the body (Table 1). Stage 1C lesions are characterized by the first appearance of a vascular fibroproliferative tissue (Fig 1, panel 1c) that surrounds and invades the contiguous skeletal muscle. Mast cells are noted in abundance at this stage with a tissue density of 4 to 15 mast cells per 10 high-power fields, 40- to 150-fold greater than the density observed in normal skeletal muscle and nonlesional FOP muscle, and 10- to 46-fold greater than that observed in any of the inflammatory myopathies (Table 1).

As an FOP lesion progresses into stage 2A (Fig 1, panel 2a), a highly monotonous vascular and edematous fibroproliferative lesion is noted. Mast cells are noted in abundance at the leading edge and around the periphery of the fibroproliferative lesion at a density indistinguishable from that of the 1C lesion. Stage 2B FOP lesions are identified by the first appearance of cartilage (Fig 1, panel 2b), and the mast cells are generally confined to the fibrous pseudocapsule surrounding the cartilage. In stage 2C, the mature stage of the FOP lesion, endochondral ossification is noted (Fig 1, panel 2c). At this stage, mast cells are noted only at the periphery of the lesion at a density that is similar to that of the early stage 1A lesion (Table 1) but still 10-fold higher than in uninvolved skeletal muscle. However, at this stage, there is no longer any residual histologic presence of muscle; the previously existing skeletal muscle tissue has been replaced by bone.

DISCUSSION

The most significant finding of our study was the dramatic mobilization and activation of inflammatory mast cells at all stages of FOP lesional development. These data document an unanticipated and previously unrecognized presence of mast cells in the pathology of FOP lesions. Mast cells have long been known to be involved in bone remodeling, but this is the first report to implicate their involvement in de novo osteogenesis. Recent studies have elucidated conditions, such as disruption of the myofiber membrane, that induce mast cell accumulation in skeletal muscle. In an injured muscle, peak mast cell accumulation occurs at the onset of regeneration, approximately 3 days after experimental phospholipase injection or 11 days after ischemic injury, but not during the phase of muscle necrosis. The pattern of mast cell infiltration in FOP lesions is qualitatively different from these findings and shows a steady progression of the mast cell numbers and association with myonecrosis and neangiogenesis. The appearance of mast cells in FOP lesions is thus qualitatively different from that seen in a variety of inflammatory myopathies and quantitatively much more intense.

Mast cells play a prominent role in the local invasion of tumors. Mast cells accumulate at the leading
edge of invading carcinomas, where they are conscripted for angiogenesis and local tumor invasion, but mast cells are not found in the core of the invading tumors. In FOP, mast cells are found in abundance predominantly at the leading edge of the more mature FOP lesions (stages 2A to 2C) and within the lesions themselves at the earlier, less mature stages (1A to 1C).

Mast cells discharge their stored granules into the interfascicular matrix of skeletal muscle upon exposure to a wide variety of immunologic or external stimuli, including thermal or mechanical trauma and T cell-mediated inflammatory processes. T cell–mast cell interactions are intimate, bidirectional, and robust. Mast cells are able to present antigens to T cells, and mast cell–derived cytokines such as interleukin-4 stimulate T cells to differentiate into various T-cell subtypes. Conversely, T cell–derived mediators directly induce mast cell degranulation. The intimate temporal and spatial relationship of lymphocytes and mast cells in the FOP lesions lends additional support to the cooperative interaction of these hematopoietically derived cell types.

Our findings provide an intriguing model for the pathophysiology of FOP lesions that builds upon our earlier knowledge (Fig 2). Cells in FOP are genetically conditioned toward a bone induction response, and lymphocytic infiltration of early FOP lesions has been observed. We hypothesize that tissue injury in FOP leads to lymphocyte migration into skeletal muscle. Subsets of lesional lymphocytes overproduce BMP4 and lead to mast cell mobilization, a finding supported strongly by the FOP pathology and by models of bone morphogenetic protein–induced heterotopic ossification (Glaser et al, manuscript in preparation). Mediators released by degranulating mast cells stimulate a re-entrant cycle of inflammatory edema, fibrosis, and angiogenesis potentiated at the leading edge of an advancing FOP lesion. Reactive fibroblasts produce stem cell factor, which leads to further proliferation of mast cells and a self-sustaining escalation of the disease process known as a flare-up. Eventually, TGF-β released by mast cells and other lesional cells regulates the lymphocytic recruitment and migration, while endogenous overexpression of BMP4 in the fibroproliferative core drives the fibroproliferative lesion toward ossification through an endochondral pathway, regardless of the presence of mast cells (Fig 2).

At present, no animal models of FOP mimic the flagrant tissue edema and the rapid spread of a preossseous fibroproliferative lesion along tissue planes. The presence of a normal skeleton in mast cell–deficient animals and the paucity of mast cells in experimental fracture healing suggest that mast cells are not necessary either for the embryonic formation and maturation of skeletal elements or for the repair of fractures. Furthermore, mast cell–deficient animals form heterotopic bone after BMP implantation (Glaser, manuscript in preparation). Just as mast cells play an important role in tissue invasion of precancerous lesions, mast cells may promote invasive osteogenic lesions such as FOP. To determine whether mast cells play a role in the spread of FOP lesions, a controlled clinical trial of mast cell inhibitors may be warranted in patients with FOP. The observation of mast cell mobilization in FOP lesions provides a novel and previously unrecognized opportunity to evaluate anti-mast cell therapies in FOP.

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REFERENCES