Bone Lineage Proteins in the Entheses of the Midfoot in Patients with Spondyloarthritis

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ABSTRACT. Objective. Patients with juvenile-onset spondyloarthritis (SpA) may develop ankylosis of the midfoot resembling the spinal changes seen in patients with ankylosing spondylitis (AS). The study of the histopathology of the feet of patients with tarsitis could help us understand the pathogenesis of bone formation in affected structures in the SpA. The objective of our study was to describe the histopathologic characteristics of the midfoot in patients with tarsitis associated with SpA.

Methods. We obtained synovial sheaths, entheses, and bone samples from 20 patients with SpA with midfoot pain/tenderness and swelling. Tissue samples underwent H&E staining; immunohistochemistry for CD3, CD4, CD8, CD68, and CD20 cell identification; and immunofluorescence for bone lineage proteins, including osteocalcin, osteopontin, parathyroid hormone-related protein, bone sialoprotein, and alkaline phosphatase.

Results. Slight edema and hyalinization were found in some tendon sheaths, and few inflammatory cells were detected in the entheses. In bones, we found some changes suggesting osteoproliferation, including endochondral and intramembranous ossification, but no inflammatory cells. In entheses showing bone proliferation, we detected osteocalcin and osteopontin in cells with a fibroblast-mesenchymal phenotype, suggesting the induction of entheseal cells toward an osteoblast phenotype.

Conclusion. Osteoproliferation and abnormal expression of bone lineage proteins, but no inflammatory infiltration, characterize midfoot involvement in patients with SpA. In this sense, tarsitis (or ankylosing tarsitis) resembles the involvement of the spine in patients with AS. Ossification may be in part explained by the differentiation of mesenchymal entheseal cells toward the osteoblastic phenotype. (J Rheumatol First Release Dec 15 2014; doi:10.3899/jrheum.140218)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS
ANKYLOSING TARSITIS
OSTEOCALCIN
OSTEOPONTIN

Spondyloarthritis (SpA) refers to a group of HLA-B27–positive rheumatic diseases that share clinical and genetic features1. Signs, symptoms, and radiographic features define the diseases and conditions that constitute the group. Inflammation of the synovium and enthesis, as well as ossification of the latter, are the pathologic processes that stand behind the clinical picture of SpA, mainly ankylosing spondylitis (AS).

In Latin American patients with SpA, the prevalence of peripheral arthritis and enthesitis is higher and occurs earlier than in whites2. A unique form of severe involvement of the feet, named ankylosing tarsitis (AT), has been described in Mexican youngsters with SpA3,4. AT consists of tarsal swelling, synovial inflammation, bone overgrowth, endochondral ossification, enthesophytes, bone bridging, and ankylosis of the tarsal bones (Figure 1). The changes that occur in this form of disease resemble those found in the radiographic and magnetic resonance imaging (MRI) studies of the spine in patients with AS.
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The scarcity of histopathologic samples, particularly of the spine and sacroiliac (SI) joints, has hampered our understanding of the pathogenesis of SpA, especially in patients with early disease at the start of the ossification. We assume that the study of the midfoot bones of patients with SpA undergoing ossification could be a complementary approach.

Both inflammation and osteoproliferation are fundamental processes to be understood.

Histopathologic studies of the spine and SI joints of patients with AS have focused primarily on inflammation; these studies have assessed the immune cell populations and also their secreted cytokines. T cells are the predominant cell found in the infiltrates (mainly the CD4, CD8, CD45RO, and CD45RA subtypes), but macrophages are also present at lower counts; inflammatory infiltrates are mainly located in the bone marrow. Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, have been detected in areas of inflammatory infiltration whereas the transforming growth factor (TGF)-β is expressed in areas of new bone formation. Local bone marrow macrophages and dendritic cells produce interleukin (IL)-23 and Th cells 17 produce IL-17.

Ossification, on the other hand, has not been studied extensively in patients with SpA despite its relevance in a patient’s prognosis and its potential as a therapeutic target. Ossification of enthesal and spinal structures in SpA involves either the enchondral or the intramembranous pathways. This ossification may result either from the proliferative response of the periosteal edges intruding into the enthesis, or from the differentiation of mesenchymal cell precursors in the neighboring tissues after an osteogenic stimulus. The intimate mechanisms behind this ossification are likely diverse to induce ossification in a diversity of cell lineages that reside in different anatomical structures (i.e., nonenthesal zygapophyseal cartilage vs entheseal intradiscal annulus fibrosus). For a tissue to become ossified, the cell precursors require osteogenic stimuli; then the primed cells undergo sequential stages of differentiation in which they express an array of stage-specific protein markers. Among those markers, some define the early stages of differentiation like bone sialoprotein (BSP) while the expression of others such as osteopontin (OPN) or osteocalcin (OCN) define latter stages, including mature osteocytes.

The expression of these markers for bone lineage commitment has not been studied in tissues undergoing ossification of the SpA. Understanding the process and the mediators for the abnormal ossification in SpA tissues could provide an additional rationale to design specific therapy against osteoproliferation.

We analyzed the histological picture of the osteoproliferation response in the midfoot bones from patients with AT, with particular interest in the expression of bone lineage commitment proteins within the ossifying tissues.

MATERIALS AND METHODS

Ours is a cross-sectional study of adult patients with undifferentiated SpA and AS with active inflammation of the tarsal region (defined as the midfoot, from the ankle to the metatarsophalangeal joints) as defined by the presence of swelling or limitation of motion accompanied by heat, pain, or tenderness for > 2 weeks. Patients with a history of congenital malformations or acquired disease, or trauma, surgical, or immobilization procedures at any time were not included in our study. Patients who had received local foot and/or intramuscular injections and/or oral glucocorticoids in the last 3 months were not included in our study. The Hospital General de Mexico review board for human research approved our study protocol. Our study was conducted according to the Declaration of Helsinki and local guidelines. All patients signed a consent form after being informed about the nature of the study, in particular the procedures and risks of the foot biopsy.

Clinical evaluation. Demographic and clinical data were obtained for all patients. The patients included in our study underwent the following assessments: the number of painful and swollen joints, enthesis and tarsal area counts for tenderness and swelling, Schober test, occiput-to-wall distance, and chest expansion. In addition, patients completed the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the Bath Ankylosing Spondylitis Functional Index (BASFI), and the Bath Ankylosing Spondylitis Patient Global Score (BAS-G) questionnaires. We also measured C-reactive protein (CRP) serum levels or the erythrocyte sedimentation rate. Radiographic studies included those of the feet as well as the SI joints; their scoring relied on the SpA-Tarsal Radiographic Index (SpA-TRI) and the modified New York AS criteria for the SI joints.

Site selection and biopsy procedures. Biopsy sites were selected by 2 of the investigators (CP-T and AU-V). We obtained tissue samples from tendon attachments at the dorsal aspect of the tarsus, specifically the talonavicular ligament, as well as the flexor tendons synovial sheaths of the toes, including those from extensor digitorum longus and brevis, extensor hallucis longus, the inferior extensor retinaculum, and the peroneus longus and brevis. Criteria for site selection included easy access to structures displaying at least 1 of the following characteristics: (1) spontaneous pain, (2) pain and/or tenderness elicited by digital palpation, and (3) soft tissue swelling.

Tissue samples were obtained under local, subcutaneous anesthesia (lidocaine 1% and epinephrine 0.005%) in a circular pattern around the biopsy site. Tissue samples were obtained either by open skin dissection with a scalpel or percutaneous soft tissue and bone biopsy using a Craig needle instrument set and under fluoroscopic guidance. We obtained fragments of tendon and its synovial sheath, enthesis-ligament or tendon attachments, bone, and whenever possible, bone marrow samples. Our initial strategy targeted the tendon sheaths insertion along the tarsal bones, but based on the preliminary histologic review, after the third patient we decided to include an additional bone biopsy; therefore, our analysis included 20 tendon sheaths and 18 bone biopsies.

Figure 1. Radiograph from a 31-year-old patient with endstage AT. Complete fusion of the tarsal joints as a result of widespread bone proliferation. The disease onset was at 17 years and inflammatory back pain started at 21 years. The patient fulfilled AS criteria at age 25. AT: ankylosing tarsitis; AS: ankylosing spondylitis.
Tissues were fixed in 4% paraformaldehyde. Bone samples were dipped and decalcified in nitric acid until suitable for sectioning. Once decalcified, all tissue samples were washed in ethanol, dehydrated in xylool, and embedded in paraffin. After sectioning, the samples were mounted on several slides for H&E staining. A pathologist (RP-T), blinded to demographic and clinical characteristics, evaluated all samples and determined the presence or absence of inflammatory infiltrates, edema, fibrosis, hyalinization, vascularization, synovitis, and bony proliferation. The pathological features of the cartilage, periosteum, and bone marrow were also described.

Immunohistochemistry of the inflammatory infiltrates was performed with specific monoclonal antibodies (DAKO) against CD3, CD4, CD8, CD20, and CD68 markers and the following protocol: after standard microwave antigen retrieval (citrate buffer at pH 6.0 for 6 min), the slides were blocked with 10% goat serum, incubated overnight at 4°C with the primary antibody [1:200 dilution in phosphate buffered saline (PBS)], washed in PBS, and then incubated with biotinylated secondary antibody (specific-complementary to the primary; 1:1000 dilution in PBS) for 1 h at room temperature. Sections were then incubated with avidin-biotin-complex/horseradish peroxidase complex for 30 min, washed with PBS, and then treated with 3,3′ diaminobenzidine (Sigma D-5637) and 0.17% hydrogen peroxide in Tris buffer; counterstaining was done with hematoxylin. Immunohistochemistry for bone markers OPN, OCN, and parathyroid hormone-related protein (PTHrP) was also performed.

Immunofluorescence was performed with monoclonal antibodies (Santa Cruz Biotechnology) against the following markers of the osteoblast lineage cells: OPN, OCN, PTHrP, and BSP. Following blocking with 10% goat serum in PBS, sections were incubated with primary antibody (1:200 dilution) in a humid chamber at 4°C overnight, washed in PBS, and then incubated at room temperature in the dark for 1 h with the isotype-specific secondary antibody in a 1:1000 dilution: fluorescein isothiocyanate to OCN and OPN, Texas red to PTHrP and cyanine 5-labeled to PTHrP. Fibroblast Marker (ER-TR7; Santa Cruz Biotechnology) was used to perform colocalizations with OPN and OCN, and a colocalization of OCN and OPN was carried out. Labeling was evaluated by epifluorescence microscopy (Zeiss AxioScope) and/or laser scanning confocal microscopy (LSM 700 Axio Observer, Carl Zeiss), and then analyzed with the Axiovision and/or Zen10 program. The same observer carried out immunofluorescence evaluation and interpretation, and the slides were photographed under conditions optimized by the software for the exposure times.

RESULTS

We included 20 patients, mostly males, with juvenile (n = 11) or adult (n = 9) onset AS (n = 7). Their mean age ± SD at the time of our study was 27.15 ± 8.9 years. Most patients complained of axial symptoms in combination with tarsal swelling and pain. At least 50% of the group had severe proliferative changes in the tarsus according to SpA-TRI (Supplementary Figure 1, available online at jrheum.org). The mean swollen joint counts and enthesitis sites were 3.2 ± 2.75 and 8.6 ± 5.4, respectively. Twelve patients were in functional class III or IV. No patient had been previously or currently treated with biologic agents. The patients were divided based on the presence or absence of bone proliferative changes. In the group with proliferation, certain tendencies were observed: they were younger at the time of the biopsy and at disease onset, had higher swollen and tender joint and enthesitis counts, and with higher BASDAI scores, the latter was the only variable achieving statistical significance (Table 1).

Histopathology. Most tissue samples corresponded to the peroneal and tibial tendons’ sheaths, and in some cases, the

| Table 1. Comparison of patient groups defined by the presence or absence of proliferation in bone biopsies. Values are mean ± SD unless otherwise specified. |
|------------------|------------------|------------------|------------------|
| Patients, n = 18 | Patients with No Proliferation, n = 9 | Patients with Proliferation, n = 9 | p      |
| Sex, M/F         | 7/2              | 6/3              | 0.45             |
| Age, yrs         | 30.78 ± 8.27     | 23.78 ± 2.68     | 0.08             |
| Age at onset, yrs| 24.78 ± 8.85     | 19.78 ± 8.30     | 0.23             |
| Adult/juvenile-onset | 5/4            | 3/6              | 0.63             |
| Disease duration, yrs | 4.30 ± 1.43   | 2.39 ± 0.79      | 0.24             |
| Swollen joint count, 0–66 | 2.56 ± 2.02   | 3.67 ± 2.95      | 0.37             |
| Entheses index   | 7.78 ± 4.96      | 10.78 ± 5.44     | 0.24             |
| SpA-TRI, ≤ 2 / ≥ 3 | 6/3              | 3/6              | 0.17             |
| BASDAI           | 3.66 ± 1.43      | 6.20 ± 1.21      | 0.004            |
| BASFI            | 3.94 ± 3.18      | 6.54 ± 2.79      | 0.155            |
| BAS-G            | 1.77 ± 0.85      | 2.5 ± 0.87       | 0.141            |

Significant data are in bold face. SpA-TRI: Spondyloarthritis-Tarsal Radiographic Index; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BAS-G: Bath Ankylosing Spondylitis Patient Global Score.

dorsal aspect of the navicular bone or the naviculocuneiform joint. In these sheets, slight inflammation accompanied by some interfibrillar edema and disorganization (Figure 2A) and loosening of collagen fibers (Figure 2B) was evident (Table 2). Some cases showed dilated vascular loops with some signs of vascular proliferation and redundancy (Figure 2C). In cases showing evidence of chronicity, there was more often sparse cellularity, a relative absence of fibroblasts, and the development of compacted tendon sheaths; this aspect was different from that found in the extracartilagenous matrix, which showed collagen fiber hyalinization (Figure 2D). In one-third of the cases, we obtained synovial tissues that showed mild inflammatory processes consisting of a slight leukocyte infiltrate (Figure 2E) with no synovial cell proliferation.

Some samples showed marked vascularity (Figure 2F) and intrusion of osteoid material within the cartilage matrix (Figure 2G), evolving in a pattern that looked similar to that of the trabecular bone, suggesting endochondral ossification.

Entheses of the tibiofibular muscles in the dorsal aspects of the navicular, cuboid, and cuneiform bones showed some irregularities at the interface of the fibrous attachment to the periosteum (Figure 2H) and some indications of cortical bone proliferation and enthesal ossification (Figure 2I), sometimes away from the cortical bone (Figure 2J, 2K, 2L). In several sections, we observed recent osteoid deposits extending directly into the enthesis. The appearance of the cortical bone aspect suggested bone apposition. Interestingly, we found no significant leukocyte infiltration in the bone narrow.

Detection of cell subpopulations by immunohistochemistry. Scarce mononuclear cell inflammatory infiltrates, mainly perivascular, were found in tendon sheaths and within the
Figure 2. Histologic images of several structures in the joints and entheses of patients with AT. Tendon sheaths presented edema with disorganization of the collagen fibers (A, B, C) and vascular proliferation (B, C). In cases with more chronic inflammation, the collagen fibers showed hyalinization (D). Synovial membrane (E) also showed high vascularity, but no significant inflammatory infiltrates. Subchondral bone (F, G) showed irregular borders with bone proliferative response and tendency to intrude in the cartilage matrix (arrows). The entheses showed blurred bone-tendon borders, disorganized fibrillar structure, and the proliferation of tenocytes and periosteal cells (H, I). Evident ossification of the enthesial tissue beyond the periosteum (I, K, L; black arrows) was detected, suggesting a transformation of the enthesial cells toward osteoblastic lineage. No significant inflammation was detected adjacent to the tissue undergoing ossification. AT: ankylosing tarsitis.

Figure 3. Immunohistochemistry photographs of enthesial biopsies in patients with AT. Bone lineage proteins (OCN, OPN, PTHrP) are expressed in both the bone (B) and in the entheses (E) with different patterns, with OCN by far the most abundant. Cellular subpopulation detection (CD3, CD4, CD8, CD20, CD68) showed a very isolated staining, suggesting a minimal presence of immune cells. A negative control (NC) is presented as a reference. AT: ankylosing tarsitis; OCN: osteocalcin; OPN: osteopontin; PTHrP: parathyroid hormone-related protein.
entheses. In all cases, the number of cells was very low to be counted (Figure 3): positive detection of isolated CD3+ cells was possible in 16% of the slides, CD4 was detected in 36%, CD8 in 20%, CD20 in 40%, and CD68 in 29% (Figure 3).

This pattern of distribution replicates the findings of Benjamin, et al, in which inflammatory cells were found infiltrating the tendon or entheses, but rather were associated with perivascular structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>Findings</th>
<th>n (%)</th>
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<tr>
<td>Tendon sheaths, n = 20</td>
<td>Edema</td>
<td>7 (35)</td>
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<tr>
<td></td>
<td>Vascular proliferation</td>
<td>9 (45)</td>
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<tr>
<td></td>
<td>Fiber hyalinization</td>
<td>7 (35)</td>
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<tr>
<td></td>
<td>Synovial lining cells</td>
<td>7 (35)</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Bone, n = 18</td>
<td>Proliferation</td>
<td>9 (50)</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>8 (44.4)</td>
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<tr>
<td></td>
<td>Combined inflammation proliferation</td>
<td>4 (22.2)</td>
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<tr>
<td></td>
<td>Bone marrow observed</td>
<td>6 (33.3)</td>
</tr>
<tr>
<td></td>
<td>Vascular proliferation</td>
<td>15 (83.3)</td>
</tr>
<tr>
<td></td>
<td>Cartilage, hypertrophic</td>
<td>7 (38.9)</td>
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Immunofluorescence studies. OCN, OPN, BSP, and PTHrP were found in the enthesal and osteal tissues of 9 patients showing bone proliferation (Figure 4). OCN is a marker of mature postproliferative cells; the labeling was strong and evident in the bone matrix, osteocytes, and osteoblasts, but particularly intense in the entheses (77%) and periosteal tissues (Supplementary Figures 2 and 3, available online at jrheum.org). OPN, which is a marker for osteoblast differentiation at early and later stages, was evident in the bone matrix, in the osteoblasts, and expressed in the entheses (55%). BSP staining was prominent in the bone matrix, osteoblasts, and osteocytes, but was inconsistently found in
the entheses (22%). PTHrP expression was absent from the bone matrix, and seen primarily in the osteoblasts and occasionally in the entheses (11%), although with a milder intensity than OCN.

We detected colocalization of OPN and OCN in several biopsies and there was a coexpression in enthesial cells, although both proteins were expressed in different regions in the bone, with OCN more abundant in the periosteal and enthesal region (Figure 5C). Finally, both OCN and OPN colocalized with a fibroblast marker in the enthesis (Figure 5D and 5E, respectively). The production of both bone lineage proteins by fibroblasts within the enthesis proves their differentiation toward osteoid precursors.

**DISCUSSION**

Our study analyzed the histopathological findings of the entheses and joints of the midfoot of patients with active SpA and characterized immune cell subpopulations osteoblastic lineage cells in the tissues. The rationale for selecting the feet for our study is based on the similarities found in the radiographic and MRI studies of patients with axial SpA, particularly AS, and patients with peripheral arthritis and enthesitis, specifically AT. The involvement of the foot in patients with SpA, particularly of those with juvenile-onset SpA, may evolve from active inflammatory stages to partial or complete joint space narrowing and bony bridging between the tarsal bones. These changes resemble those found in the SI joints of patients with AS, including subchondral bone fusion and enthesophyte formation as consequences of intramembranous ossification of the entheses.

Interestingly, we found only scarce mononuclear cell inflammatory infiltration, and mainly CD20+ and CD4+ cell subtypes, in the synovium of tendon sheaths and in the vascular structures adjacent to the entheses. CD4 rather than CD8 T cell subtypes predominated in a previous study of the SI joints, which also found TNF-α and TGF-β in inflammatory and bone areas of the SI joints, respectively. Francois et al\(^7\) and Appel, et al\(^5\) found CD3+ T cells, and less frequently CD8+ cells, in bone marrow inflammatory infiltrates of patients with both early and longstanding AS.

Higher cell counts in the bone marrow infiltrates have been correlated with MRI findings\(^6\); patients with a higher activity score in MRI also exhibit higher inflammatory cell counts in SI joint biopsies.

We did not find any significant accumulation of inflammatory cells in the bone marrow of the tarsal bones. This finding could be explained by the fact that the bones where bone edema and inflammation are detectable by MRI are the large ones (calcaneus and astragalus) and the signal is weaker in cuneiforms and navicular. Nevertheless, the latter do indeed exhibit bone bridging and ankylosis. While most animal models of SpA develop important mononuclear cell infiltration, including in the tarsal region before ossification of the enthesis becomes clear\(^22\), enthesophytosis and bone ankylosis develop in the absence of bone marrow inflammation in the ankylosing enthesopathy murine model of SpA.

One of the most interesting findings in our study suggests the possibility of intramembranous ossification of the enthesis and subchondral osteoproliferation, with the enthesal cells expressing osteoblast-associated proteins in patients with bone proliferative changes in the absence of extensive inflammation. Both OPN and OCN were expressed in cells with fibroblastic (mesenchymal) phenotype in the fibrous entheses, whereas a more restricted-to-the-bone distribution was observed for both BSP and PTHrP. It is worth noting that OPN is expressed in a variety of cell types and participates in remodeling of normal tissues and tumorigenesis.\(^23\) It is, however, also a marker of the osteoblasts in normal and pathological calcification processes, including atherosclerosis.\(^24,25,26,27\) OCN is a noncollagenous vitamin K-dependent protein expressed and secreted in the late stage of osteoblast differentiation.\(^13\) While some evidence supports a role for OCN in bone mineral maturation, considerable effort is going toward determining its precise role in carboxylated or noncarboxylated forms in bone and other tissues.\(^28\) Although OCN is not constitutively expressed on normal tendons, it is upregulated in tendons undergoing abnormal ossification.\(^25,29,30,31\)

Interestingly, the identification of OCN in the fibrous entheses of the tarsal joints in our study suggests a role for this protein in AT. The absence of a direct comparator limits our interpretation significantly. However, we have clinical and imaging evidence of abnormal ossification of the tarsal joints in these patients, and some molecular mechanisms should account for it. OCN is expressed in tissues undergoing abnormal ossification; it was in the entheses of our patients. It must be mentioned, however, that only half of the patients with AT had proliferation-suggestive lesions, and half had inflammatory infiltrates.

Indeed, this link between inflammation and bone formation remains an unresolved issue in SpA. Inflammation and tissue damage constitute the initial insults, whereas healing and repairing are the subsequent processes that bring about, by independent mechanisms such as mechanical factors, the reengineering of the bone. MRI studies have shown some evidence that inflammation (defined as bone edema) at the vertebral corner precedes syndesmophyte formation in anti-TNF–naïve and anti-TNF–treated patients even though the effect of anti-TNF agents impedes the progression of the disease.\(^32,33,34\) In addition, it seems that the prognosis of healed lesions differs from those showing fat infiltration, but the risk for syndesmophyte formation is about the same. Radiographic progression has been associated with high CRP levels, BASDAI, and bone inflammation on MRI\(^35\).

The study of animal models documents the relationship
between inflammation and bone proliferation markers. Collagen I and III, BSP, and OCN upregulation has been described in the proteoglycan-induced spondylitis murine model. Additionally, Lories, et al demonstrated a potential role for the bone morphogenetic proteins in the entheseal ossification and also a theoretical connection with mechanosensing proteins of the family Wingless Int (Wnt). Aberrant Wnt signaling was also observed in the spontaneous ankylosis seen in ank/ank mice, which like the tissues in our present study, reveals little inflammation at site of ankylosis.

The decalcifying process interfered with mRNA and cytokine transcript identification. However, the scarcity of inflammation in our samples did not warrant any significant cytokine identification. In contrast, we identified bone lineage proteins in our tissue samples. We found no correlation between clinical findings on examination and histopathological findings. Bone, tendon, and entheses tenderness did not correlate with histologic findings in many cases.

We report an osteoproliferation process in patients with severe involvement of the tarsal joints and entheses in patients with AT associated with SpA. Our findings suggest the differentiation of entheseal cells toward an osteoblastic lineage and the formation of bone islets embedded within the enthesis matrix. The scarcity of inflammation infiltration suggests that osteoproliferation in the tarsal region might result from a predominantly mechanical rather than an inflammatory phenomenon. Because most patients developing AT have juvenile-onset disease, it is possible that bone growth factors might also play a role in this form of SpA.

ONLINE SUPPLEMENT
Supplementary data for this article are available online at jrheum.org.

REFERENCES


