Inflammatory bowel disease causes reversible suppression of osteoblast and chondrocyte function in mice

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Departments of 1Physiology, 2Radiology, and 3Pathology, 4Cell and Molecular Biology Program, 5Center for Integrated Toxicology, and 6Biomedical Imaging Research Center, Michigan State University, East Lansing, Michigan; and 7Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan
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Harris L, Senagore P, Young VB, McCabe LR. Inflammatory bowel disease causes reversible suppression of osteoblast and chondrocyte function in mice. Am J Physiol Gastrointest Liver Physiol 296: G1020–G1029, 2009. First published March 19, 2009; doi:10.1152/ajpgi.90696.2008.—Decreased bone density and stature can occur in pediatric patients with inflammatory bowel disease (IBD). Little is known about how IBD broadly impacts the skeleton. To evaluate the influence of an acute episode of IBD on bone, 4-wk-old mice were administered 5% dextran sodium sulfate (DSS) for 5 days to induce colitis and their recovery was monitored. During active disease and early recovery, trabecular bone mineral density, bone volume, and thickness were decreased. Cortical bone thickness, outer perimeter, and density were also decreased, whereas inner perimeter and marrow area were increased. These changes appear to maintain bone strength since measures of moments of inertia were similar between DSS-treated and control mice. Histological (static and dynamic), serum, and RNA analyses indicate that a decrease in osteoblast maturation and function account for changes in bone density. Unlike some conditions of bone loss, marrow adiposity did not increase. Similar to reports in humans, bone length decreased and correlated with decreases in growth plate thickness and chondrocyte marker expression. During disease recovery, mice experienced a growth spurt that led to their achieving final body weights and bone length, density, and gene expression similar to healthy controls. Increased TNF-α and decreased IGF-I serum levels were observed with active disease and returned to normal with recovery. Changes in serum TNF-α (increased) and IGF-I (decreased) paralleled changes in bone parameters and returned to normal values with recovery, suggesting a potential role in the skeletal response.

Inflammatory bowel disease (IBD) affects nearly 1.4 million people in the United States and is the most common chronic gastrointestinal illness in children and adolescents. The two major forms of IBD, Crohn’s disease (CD) and ulcerative colitis (UC), are risk factors for bone loss in adults (6, 8, 10, 15, 23, 54, 58–60). Bone pathologies during childhood can have long-term effects since this is the key period of life when maximal bone density is attained. Consistent with decreased bone density, reports indicate increased fracture risk in children and adults with IBD (55, 65). Both male and female IBD patients are affected (15) although the magnitude of bone loss may have a gender component (2).

Bone density and growth are determined by the combination of anabolic osteoblast activity (bone formation) and catabolic osteoclast activity (bone resorption), as well as the activity of chondrocytes (cartilage formation needed to extend bone length). During normal bone growth, chondrocytes continually mature and produce cartilage at the growth plate to allow bones to lengthen (endochondral bone formation). Osteoclasts resorb the cartilage and areas of existing bone and osteoblasts form new bone. These processes are regulated at multiple steps. Osteoclasts are derived from hematopoietic stem cells that progress along the monocyte/macrophage lineage pathway. In response to stimuli, such as RANKL (receptor activator for nuclear factor-κB ligand), preosteoclast differentiation can be promoted which is marked by expression of tartrate resistant acid phosphatase (TRAP5), a protein involved in the breakdown of bone. Osteoblasts are derived from pluripotent mesenchymal bone marrow cells. Their activity is regulated at three key steps: lineage selection, differentiation, and viability. Lineage selection is a key area of regulation since mesenchymal stem cells can also give rise to other cells including chondrocytes and adipocytes. It is thought that selection of one lineage could potentially be at the cost of another; this is suggested in conditions of bone loss (such as aging, type 1 diabetes, and disuse-associated osteoporosis) in which osteoblast function is decreased and bone marrow adiposity is increased (1, 36, 44, 67). Expression of the transcription factors Runx2, Sox9, and PPARy2 promotes osteogenesis, chondrogenesis, and adipogenesis, respectively. Maturation of osteoblasts, chondrocytes, and adipocytes is marked by expression of several genes including osteocalcin, collagen II and X, and fatty acid binding protein 4 (aP2), respectively. Therefore examination of maker gene expression contributes to the assessment of a disease or condition’s impact on these cells.

To develop and/or optimize treatments for bone loss and decreased growth stature in children with IBD, the mechanisms that contribute to the bone pathology must be identified. Studies in humans are confounded by interfering actions of therapies used to treat IBD and by the lack of bone histology and architecture studies needed to accurately assess osteoblast, chondrocyte, adipocyte, and osteoclast changes as well as trabecular and cortical bone adaptations. To address this, we induced IBD pharmacologically [5% dextran sodium sulfate (DSS) orally for 5 days] in mice during their linear skeletal growth phase. DSS is thought to induce colitis through its toxic effect on intestinal cells, which leads to a breach in the mucosal barrier and subsequent exposure to luminal antigens leading to inflammation (31, 69). Although DSS induced intestinal pathology has proven similarities to UC, marked by focal crypt lesions and mucosal inflammation (45), it also exhibits some features of CD such as submucosal inflammation and ulceration. Our findings demonstrate that mice treated with DSS exhibit significant changes in bone, marked by decreased
stature and bone loss. Osteoblast, chondrocyte, and adipocyte activities and differentiation states were all suppressed during active disease. These changes were restored with overt recovery from active disease.

MATERIALS AND METHODS

Mouse model. Four-week-old C57BL/6 male mice were obtained from Harlan (Indianapolis, IN). Animals were housed in a 12:12-h light-dark cycle at 23°C in groups of up to five animals per cage with autoclaved bedding. Food and water were given ad libitum. Body weights and food and water intakes were monitored regularly. IBD was induced by adding 5% DSS (36,000–50,000 molecular weight; catalog no. 160110; MP Biomedical LLC; Solon, OH) to the sterile Milli-Q drinking water of the mice for 5 days then removed for 2, 10, 24, and 38 days; this represents 7, 15, 29, and 43 days following first day of DSS administration.

At each time point, terminal body, fat pad, and muscle mass were recorded. Vertebrae, calvaria, tibias, and femurs were removed and cleaned of muscle and tendon before processing. Bones were either fixed in 10% neutral buffered formalin for histological processing and analyses or snap frozen in liquid nitrogen and stored at −80°C. To confirm IBD induction, cecum and colon were collected. A 2-cm piece of the proximal end of the ascending colon was snap frozen and stored at −80°C for RNA analyses. The cecum and transverse colon through anus were fixed and stored as described. Serum was collected from coagulated blood, spun 10 min at 4,000 rpm, and stored at −80°C. Serum osteocalcin (catalog no. BT-470; Biomedical Technologies, Stoughton, MA), active TRAP5b (IDS osteocalcin assay; Immunodiagnostic Systems, Fountain Hills, AZ), IGF-I (mouse IGF-I ELISA; AssayPro, St. Charles, MO) and TNF-α [TNF alpha kit (mouse) EIA; Alpco Diagnostics, Salem, NH] levels were measured according to manufacturer protocols. All animal protocols were reviewed and approved by Michigan State University Institutional Animal Use and Care Committee.

Cecum histology. Paraffin-embedded cecum sections were obtained and stained with hematoxylin-eosin as previously described (71). Active disease was evaluated by the degree of gland distortion, severity of mucosal inflammation or ulceration, mucosal thickness, and number of submucosal lymphoid aggregates. The scoring system was developed based on previous reports (11, 50, 69). Specifically, gland distortion measurements were obtained at the ileocecal junction and were scored as follows: 0, straight crypt direction; 2, crypt direction distortion; and 4, complete loss of crypt structure (gland dropout). Disease severity was examined for the entire cecum region and was scored as follows: 0, normal; 1, mild with small focal area (extending 1–5 crypts along the mucosal length) of disease and/or inflammation limited to the lamina propria; 2, moderate with multiple small focal areas of disease and/or inflammation infiltrating into the submucosa; 3, severe with large ulcers extending less than 20 crypts along the mucosal length; and 4, marked with large ulcers more than 20 crypts in length. Total mucosal thickness (height) was measured as the sum of crypt height (defined from top of epithelial cells to bottom of crypt) and submucosal height (defined from lowest base of crypt to top of muscularis). The number of lymphoid aggregates (10 or more immune cells congregated together) was counted within the submucosal layer of the cecum.

Femur histomorphometry. Fixed femurs were decalcified and processed as previously described (35). The distal femur region was evaluated for growth plate thickness (by use of safranin O stain), adipocyte number as previously described (35) and osteoblast and osteoclast surface area. For the latter, slides were examined for TRAP activity, according to manufacturer protocol (Sigma Aldrich, St. Louis, MO). Trabeculi within 0.2 mm of the proximal side of the growth plate were examined. Measurements were done with Image Pro software (version 4.5.1.22; Media Cybernetics).

MAR. Mice were injected intraperitoneally with 200 μl of filter sterilized saline containing 10 mg/ml calcein (Sigma, catalog no. C0875). Injections were given at 5 days and again at 2 days prior to harvest. Lumbar vertebrae were harvested and fixed, and undecalcified sections were cut into 5-μm sections. Distance between fluorescent calcein lines in L3 vertebrae were measured using Image Pro Plus (version 4.5.1.22; Media Cybernetics).
and divided by the number of days between injections to determine mineral apposition rate (MAR) values.

**μCT bone imaging.** Fixed bones were scanned by using a GE Explore Locus microcomputed tomography (μCT) system at a voxel resolution of 20 μm obtained from 720 views as previously described (35). Cortical bone analyses were made in a defined 2 × 2 × 2 mm cube in the middiaphysis immediately proximal to the distal tibial-fibular junction, with the exception of cortical bone mineral content (BMC) and density (BMD), which were made in a 0.1 × 0.1 × 0.1 mm cube. Trabecular bone analyses utilized a region of trabecular bone defined at 0.17 mm distal to the growth plate of the proximal tibia. Cortical BMC, BMD, moment of inertia (MOI), thickness, perimeter, and area, and trabecular BMC, BMD, bone volume fraction (BVF), and thickness values were computed by a GE Healthcare MicroView software application for visualization and analysis of volumetric image data. Cortical isosurface images were taken from a care MicroView software application for visualization and analysis of fraction (BVF), and thickness values were computed by a GE Health

**RNA analysis.** Frozen tibias were crushed under liquid nitrogen conditions prior to RNA extraction. Pulverized bones and ascending colon pieces were homogenized in TRI reagent solution (Molecular Research Center, Cincinnati, OH) and RNA extracted. RNA integrity was confirmed by formaldehyde-agarose gel electrophoresis. Real time synthesis of cDNA from RNA (2 μg) was accomplished using the Superscript II kit with oligo dT primers per manufacturer protocol (Invitrogen, Carlsbad, CA). cDNA (1 μl) was amplified by PCR in a final volume of 26 μl by using the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) with 20 pmol of each primer (Integrated DNA Technologies, Coralville, IA). Primers for gene amplification by real-time RT-PCR (iCycler, Bio-Rad) were designed or previously described: runx2, osteocalcin, cathepsin K, fatty acid binding protein 4 (aP2), and PPAR

**RESULTS**

To examine the effect of active IBD and its recovery on bone health, 4-wk-old, male, C57BL/6 mice were treated with 5% DSS from day 0 to day 5, and acute and long-term bone pathologies were examined at days 7, 15, 29, and 43. Induction of IBD by DSS treatment was confirmed by cecum histology.

<table>
<thead>
<tr>
<th>Table 1. Harvest organ masses from C57BL/6 mice</th>
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<tr>
<td></td>
</tr>
<tr>
<td>Femoral fat pads</td>
</tr>
<tr>
<td>Tibialis</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Brain</td>
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All values are expressed in grams, average ± SE; n > 5. DSS, dextran sodium sulfate. *P < 0.05.
decrease). This suggests that early on fat, protein, and glycogen are being depleted from the body at the cost of tissue mass, but that this loss is fully recoverable. Interestingly, brain mass was protected from these negative effects and remained unchanged throughout the study (Table 1).

Next, we examined whether a single bout of IBD could have an impact on bone. μCT of the proximal tibia trabecular region (adjacent to the growth plate) demonstrated a significant decrease in BMC, BMD, and BVF in DSS-treated compared with control mice at 7, 15 and 29 days (Fig. 2, A and B, Table 2). Changes were still apparent when corrected for the lower body weights of the DSS-treated mice (data not shown). Examination of reconstructed three-dimensional images of the trabecular region over the experimental time course suggest the reduction in bone volume is likely due to reduced bone growth (rather than bone loss) in DSS-treated compared with control mice (Fig. 2B). Further support for a reduction in bone growth and formation comes from increased MARs measured at day 15 and decreased tibial lengths at 15 and 29 days (Table 2). However, the significant decreases seen in bone volume, length, and other parameters were completely recovered by 43 days (Fig. 2 and Table 2).

We also examined cortical parameters of the DSS-treated mouse tibia diaphysis and found significant decreases at 15 and 29 days in cortical thickness, outer perimeter, cortical area, and density (Table 3). Inner perimeter and marrow area were higher compared with controls. The changes in bone architecture appeared to aid in maintaining bone strength since measures of moments of inertia did not differ between control and DSS-treated mice (Table 3). Similar to trabecular bone, recovery by day 43 was observed for most cortical bone parameters (Table 3) with cortical outer perimeter increasing to greater than control values (perhaps overshooting normal values) at 43 days.

Changes in bone can be site specific, so other bones were also examined. Femur, another long bone, exhibited reduced bone volume and recovery in DSS-treated mice (data not shown) similar to tibia. In vertebrae, significant decreases in trabecular bone volume fraction were observed and, unlike the long bones, vertebral bone volume fraction was not recovered by 43 days (Fig. 3). However, there was a trend toward recovery since BVF was less than 20% of control values at day 15 whereas it increased to more than 65% of control values at day 43. In contrast to long bones and vertebrae, calvaria (an unloaded, intra-membranous formed bone) only showed a trend toward decreased density at 15 and 29 days and actually exhibited an increase in density compared with control mice at 43 days. Variations between bone sites may be the result of site-specific differences in growth activity (osteoblast activity) present prior to disease onset.

To examine the cellular contribution to the DSS-induced bone loss, we measured tibial RNA and serum markers of key cell types involved in bone growth and density regulation. First, we examined markers of osteoblast maturation and activity. Runx2 (lineage selection and maturation marker), alkaline phosphatase (mild differentiation marker), and osteocalcin (late-stage differentiation marker) mRNA levels were all decreased at days 7 and 15 in DSS-treated mice compared with controls (Fig. 4A). At 29 and 43 days differences were no longer apparent, consistent with bone density and growth recovery. Measurement of percent osteoblast surface per total bone surface and measurement of serum osteocalcin levels also indicate a decrease in osteoblast activity at 7 days, but not at 15 days (Fig. 4B), suggesting that functional recovery precedes recovery at the gene expression level. These findings indicate an osteoblast dysfunction and are supported by the decrease in MAR found at day 15 (Table 2), which represents mineral laid

### Table 2. Tibial trabecular bone parameters

<table>
<thead>
<tr>
<th>Time, days</th>
<th>Group</th>
<th>Thickness, mm</th>
<th>Inner Perimeter, mm</th>
<th>Outer Perimeter, mm</th>
<th>Marrow Area, mm²</th>
<th>Cortical Area, mm²</th>
<th>BMD, mg/ml</th>
<th>MOI, mm⁴</th>
</tr>
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<tbody>
<tr>
<td>7</td>
<td>Control</td>
<td>2.41±0.03</td>
<td>3.30±0.07</td>
<td>0.42±0.01</td>
<td>0.35±0.02</td>
<td>803±29</td>
<td>0.090±0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DSS</td>
<td>2.30±0.06</td>
<td>3.09±0.06*</td>
<td>0.38±0.02</td>
<td>0.31±0.02</td>
<td>774±13</td>
<td>0.082±0.004</td>
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</tr>
<tr>
<td>15</td>
<td>Control</td>
<td>2.26±0.05</td>
<td>3.36±0.07</td>
<td>0.37±0.02</td>
<td>0.42±0.01</td>
<td>871±15</td>
<td>0.097±0.007</td>
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<tr>
<td></td>
<td>DSS</td>
<td>2.47±0.05*</td>
<td>3.21±0.06*</td>
<td>0.44±0.02*</td>
<td>0.28±0.01*</td>
<td>767±25*</td>
<td>0.101±0.006</td>
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</tr>
<tr>
<td>29</td>
<td>Control</td>
<td>2.16±0.03</td>
<td>3.47±0.06</td>
<td>0.33±0.01</td>
<td>0.50±0.01</td>
<td>982±29</td>
<td>0.095±0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DSS</td>
<td>3.21±0.01*</td>
<td>3.32±0.04*</td>
<td>0.39±0.01*</td>
<td>0.40±0.01*</td>
<td>856±18*</td>
<td>0.088±0.004</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Control</td>
<td>2.11±0.05</td>
<td>3.44±0.04</td>
<td>0.32±0.02</td>
<td>0.53±0.01</td>
<td>945±28</td>
<td>0.090±0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DSS</td>
<td>2.28±0.04*</td>
<td>3.65±0.07*</td>
<td>0.37±0.01*</td>
<td>0.56±0.03</td>
<td>999±33</td>
<td>0.101±0.007</td>
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Values are averages ± SE, n > 5. MOI, moments of inertia. *P < 0.05.
down between days 6 and 13 and is consistent with functional measures of osteoblasts.

Osteoclast markers and function were examined next since increased resorption can also contribute to bone loss. Interestingly, we observed a significant decrease in osteoclast markers, TRAP5, and cathepsin K, at 7 days and for TRAP5 also at 15 days (Fig. 5A). Osteoclast surface per total bone surface was decreased at 7 days but was increased at 15 days in DSS-treated compared with control tibias (Fig. 5B). This increase could represent osteoclasts that are not fully mature/active. This is supported by suppressed serum active TRAP5b levels, a sensitive marker of bone resorptive activity, at both 7 and 15 days, consistent with TRAP5 mRNA levels in bone.

These findings suggest that a decrease in osteoblast activity and bone formation is principally responsible for the decrease in bone volume fraction in mice with IBD. Because of the often-observed reciprocal relationship between osteoblasts and marrow adiposity, adipocyte numbers and marker levels were examined in DSS-treated and control mice. Surprisingly, marrow adiposity and adipocyte markers (PPARγ2 and aP2) were either unchanged or decreased in DSS-treated mice (Fig. 6).

Because we identified a decrease in bone length in the DSS-treated mice, chondrocyte activity and markers were also examined. As expected, we observed a decrease in growth plate thickness in DSS-treated mice at 7 days (Fig. 7A). This was fully restored by day 15, suggesting that growth plate activity was only suppressed during the period of active disease. At the molecular level, collagen II and X were suppressed at days 7, 15, and 29 (Fig. 7B), implying (as with osteoblasts) that molecular recovery can take longer than functional recovery and/or that molecular markers are more sensitive indicators of bone changes. Still, by 43 days all chondrocyte parameters return to the levels of healthy control mice.

To begin to address potential mechanisms for the suppression in osteoblast and chondrocyte function, we examined the levels of serum TNF-α and IGF-I. The former is a key cytokine demonstrated to be elevated during active IBD, and the latter is a key hormone involved in the regulation of bone density and growth. Figure 8 demonstrates that serum TNF-α levels are significantly elevated immediately following acute DSS treatment (7-day time point) and then return to normal as mice recover. IGF-I serum levels, in contrast, are significantly decreased at 7 days but similar to TNF-α levels they return to normal levels with disease recovery.

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**Fig. 3.** IBD-related bone loss also seen in vertebrae but not calvaria. Similar to long bone, decreases in lumbar (L3) vertebrae trabecular bone volume fraction were observed in DSS-treated (solid columns) compared with control (open columns) mice. Calvaria bone mineral density (BMD), however, did not change except for an increase at 43 days in DSS mice. Values represent averages ± SE; n = 5 per condition; *P < 0.05 2-tailed Student’s t-test.

**Fig. 4.** Osteoblast markers and activity are decreased during active IBD. A: expression levels of markers of osteoblast maturation: runx2, alkaline phosphatase (AP), and osteocalcin (OC) were examined by real-time RT-PCR analysis using RNA isolated from whole tibias of DSS-treated (◆) or control (○) mice. HPRT, hypoxanthine-guanine phosphoribosyl transferase. B: percentage of osteoblast surface compared with total trabecular bone surface in the metaphysis region of the distal femur of DSS-treated (solid bars) and control (open bars) mice was determined by histological staining and analyses. Further confirmation of changes in osteoblast activity was determined by ELISA quantitation of serum osteocalcin levels. Values represent averages ± SE; n ≥ 5 *P < 0.05 2-tailed Student’s t-test.
DISCUSSION

Decreased bone growth and density are often observed in children afflicted with IBD and both children and adults with IBD are at increased risk for fractures (6, 8, 10, 15, 23, 49, 54, 55, 58–60, 64, 65). Little is known about the mechanisms accounting for the bone pathology and, in particular, the role of chondrocytes (growth plate activity) and osteoblasts (bone formation) in the bone changes. Here we examined the effect of an acute treatment (5 days) with 5% DSS, and recovery from this treatment, on the function and maturation of osteoblasts and chondrocytes as well as osteoclasts and adipocytes. Functional and maturation parameters were also decreased during an acute episode of active disease. However, as mice recovered bone parameters returned to normal values with recovery, suggesting a potential role in the skeletal response.

Our studies demonstrate that during active disease serum osteocalcin levels, MAR and trabecular BVF decrease. A decrease in the number of osteoblasts and, as indicated by RNA analyses, decreased runx2 and osteocalcin RNA expression support a role for the loss of osteoblasts and suppressed osteoblast maturation contributing to IBD bone loss. Consistent with our findings of decreased MAR, Dresner-Pollak et al. (17) and Hamdani et al. (24) found bone loss and decreased mineralizing surfaces and formation rates in IL-10-deficient mice with colitis and DSS-treated mice, respectively. In our study, osteoclast activity was decreased as indicated by a suppression of multiple parameters, including osteoclast RNA markers, serum markers, and osteoclast surface. We did observe an increase in osteoclast surface at day 15, although we did not observe any increase in other osteoclast markers; this may represent a recruitment of osteoclasts (that may not be as active) during the recovery of endochondral growth. A recent report (24) also demonstrates an increase in osteoclast number in mice with colitis at 2 wk; however, no additional osteoclast parameters and returned to normal values with recovery, suggesting a potential role in the skeletal response.

Fig. 5. Osteoclast markers and activity are decreased during active IBD. A: expression levels of osteoclast markers tartrate-resistant acid phosphatase 5 (TRAP5) and cathepsin K were examined by real-time RT-PCR analysis using RNA isolated from whole tibias of DSS-treated (●) or control (○) mice. B: percentage of osteoclast surface compared with total trabecular bone surface in the metaphysis of the distal femur of DSS-treated (solid bars) and control (open bars) mice was determined by histological staining and analyses. Further confirmation of changes in osteoclast activity was determined by ELISA quantitation of serum levels of the active form of TRAP5b. Values represent averages ± SE; n = 5 *P < 0.05 2-tailed Student’s t-test.

Fig. 6. Marrow adiposity is decreased in DSS-treated mice. A: representative images of trabecular regions of distal femurs isolated at 43 days demonstrating the presence of adipocytes (large light-colored cells; 1 is noted with an arrow). B: quantification of adipocytes in the trabecular marrow region of DSS-treated (●) and control (○) mice. Additional analyses of adipocyte gene expression [examined by real-time RT-PCR using HPRT as a housekeeping gene] support a reduction in adiposity in DSS-treated mice. Values represent averages ± SE; n = 5 *P < 0.05 2-tailed Student’s t-test.
parameters were measured. Osteoblasts and osteoclasts can positively signal each other through various signaling pathways including RANKL (an activator of osteoclasts) and OPG (osteoprotegrin; a decoy receptor that inhibits osteoclast activation). We were unable to observe an alteration in RANKL and OPG levels, in contrast to some reports indicating an increase RANKL levels during colitis (4). Differences between studies may stem from differences in the colitis models and the use of IL-2 knockout mice, which may have additional effects on osteoclast activation. This would be consistent with differences in bone effects between CD and UC, where bone resorption may be more active in UC compared with CD patients (2).

Unexpectedly, we observed that the C57BL/6 mice in our experiment lost a significant amount of weight within 7 days of treatment (15% compared with their starting weight). The animals were not terminated at this point as intended, which allowed us to observe the immediate recovery response beginning at day 7, which lead to mouse body weights increasing to reach control levels by day 28. The mice exhibited decreased fat and muscle mass during active disease as seen in previous reports (37); however, we also found that these parameters were restored as mice regained health. A similar response was seen with regard to bone density and was reported in another colitis model (induced by TNBS), which demonstrated increased bone formation following healing and full restoration of bone density by 12 wk into the experiment (30). The significant loss of weight, fat, and muscle are suggestive of potential nutritional deficiencies and altered hormonal levels. Malnourishment, due to decreased absorption and/or intake, as sometimes seen in IBD patients (7), can contribute to a decrease in anabolic processes such as bone formation and growth. Reduced intestinal absorption of calcium has been considered a potential contributor to bone loss in IBD (29, 47). However, one would expect an increase in osteoclast activity (in an attempt to raise serum calcium levels) under these conditions, and we did not observe this in our studies.

The loss of fat pad mass in DSS-treated mice can also have an active role in the regulation of bone density. It is known that factors secreted by adipocytes (adipokines) such as leptin and adiponectin can contribute to the regulation of bone remodeling and linear growth (21, 39, 51). In the DSS mice, the loss of fat would suggest that the levels of these factors are reduced. Consistent with this, we observe a decrease in serum leptin levels in DSS-treated mice (data not shown), which could contribute to the observed changes in bone. In addition, DSS treatment and gut inflammation could lead to altered levels of hormones involved in the regulation of body composition and bone density. For example, ghrelin (a hormone produced pre-
dominantly by cells in the stomach) can affect body composition, modulate immune response, and increase bone density (16, 20, 62), and its serum levels are demonstrated to be altered in IBD patients (5). However, ghrelin levels are most likely not involved in IBD-induced bone loss since they are increased in IBD patients (5) and if anything should benefit bone density (16, 20, 62).

Although human studies show that IBD can stunt growth (leading to reduced height) (15, 60), to our knowledge this is the first report demonstrating that IBD induces changes in growth plate thickness and in chondrocyte marker gene expression. The reduced growth plate thickness observed at day 7 but not at day 15 or beyond indicates that bone growth may rapidly respond to disease via suppression of chondrocytes activity marked by reduced matrix gene expression (collagen II and X), but bone growth can exhibit an equally rapid recovery. A previous study in hypothyroid rats also demonstrates a rapid increase in growth plate thickness upon disease (growth plate inhibition) correction (33). This response is termed “catch-up” growth and is consistent to what we observe in our mice. Sox9, an early chondrocyte lineage selection marker, was not significantly decreased throughout most of the study (except at day 29), suggesting that IBD may target the process of chondrocyte maturation rather than lineage selection to cause suppressed endochondral bone growth. We also found that serum levels of IGF-I, a well-known positive regulator of bone growth and density (27, 43, 70), were decreased in DSS-treated mice. This is consistent with previous studies indicating relationships between decreased serum IGF-I levels and active IBD in humans (18, 28, 56). Given its anabolic effect on bone, the suppression of IGF-I levels may contribute to bone loss in IBD. Further studies will be needed to confirm this hypothesis.

Cytokines are known to be elevated in the serum of IBD patients (3, 14, 19, 32, 42, 46, 52, 53, 61, 63) and are known to influence bone and cartilage formation. Treatment of rat bone organ cultures or primary osteoblast cultures with serum from CD patients can decrease osteoblast function (26, 61, 66). Our studies demonstrate an elevation in serum TNF-α levels, which is particularly pathological cytokine, capable of causing a decrease in osteoblast maturation and an increase in osteoblast apoptosis (22, 25, 41). Similarly, IL-1β and TNF-α causes a significant impairment of bone growth in vitro marked by decreased chondrocyte proliferation and increased apoptosis (34). Furthermore, treatment of adult and pediatric CD patients with infliximab, an inactivating antibody to TNF-α, successfully treats CD and improves BMD in CD patients (9, 38, 46, 48, 57); this could be a direct effect on osteoblasts or an indirect effect through decreasing disease severity.

Taken together, these findings demonstrate that IBD affects multiple cells in the bone including osteoblasts, osteoclasts, adipocytes, and chondrocytes. Bone loss was observed at multiple sites including long bones and vertebrae. Our data also show that there are rapid responses to disease activity, both pathological as well as recovery. As mice regained heat, there were notable increases in growth and bone density until attaining or surpassing healthy control levels. Active IBD is known to be associated with an elevation in systemic cytokine levels (3, 14, 19, 32, 42, 46, 52, 53, 61, 63), which could in turn be important for orchestrating the bone disease and recovery response. We identified that serum levels of TNF-α are elevated, whereas IGF-I levels are suppressed, during the period of active IBD and bone loss. With disease recovery, TNF-α and IGF-I serum levels and bone pathology return to normal, suggesting a role for inflammation and IGF-I in IBD suppression of bone growth and density. Future studies are needed to further understand the link between IBD and its bone pathology.

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GRANTS

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