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Original Research

Age-Related Differences in Collagen-Induced Arthritis: Clinical and Imaging Correlations

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Arthritis is among the most common chronic diseases in both children and adults. Although intraarticular inflammation is the feature common among all patients with chronic arthritis there are, in addition to age at onset, clinical characteristics that further distinguish the disease in pediatric and adult populations. In this study, we aimed to demonstrate the utility of microCT (μCT) and ultrasonography in characterizing pathologic age-related differences in a collagen-induced arthritis (CIA) rat model. Juvenile (35 d old) and young adult (91 d old) male Wistar rats were immunized with bovine type II collagen and incomplete Freund adjuvant to induce polyarthritis. Naïve male Wistar rats served as controls. All paws were scored on a scale of 0 (normal paw) to 4 (disuse of paw). Rats were euthanized at 14 d after the onset of arthritis and the hindpaws imaged by μCT and ultrasonography. Young adult rats had more severe signs of arthritis than did their juvenile counterparts. Imaging demonstrated that young adult CIA rats exhibited more widespread and severe skeletal lesions of the phalanges, metatarsals, and tarsal bones, whereas juvenile CIA rats had more localized and less proliferative and osteolytic damage that was confined predominantly to the phalanges and metatarsals. This report demonstrates the utility of imaging modalities to compare juvenile and young adult rats with CIA and provides evidence that disease characteristics and progression differ between the 2 age groups. Our observations indicate that the CIA model could help discern age-related pathologic processes in inflammatory joint diseases.

Abbreviations: μCT, microCT; CIA, collagen-induced arthritis.

Arthritis is among the most common diseases in both children and adults. In children, growth, hormonal changes, and neuroimmune responsiveness and plasticity might confer influences on arthritic processes not seen in adults. Age-dependent outcomes have been demonstrated by using animal models of osteoarthritis;[5,6,11] however, there is limited information about how pathogenic processes vary among different age groups with experimental inflammatory arthritis in general[7,10] and in the collagen-induced arthritis (CIA) model of arthritis in particular.[8] Studying and comparing features of the CIA model in growing compared with mature rats can be undertaken in ways that are not possible in humans and could help to understand age-related pathologic differences in children and adults with inflammatory joint diseases. Therefore, we undertook to compare clinical and imaging features of CIA in juvenile (growing) compared with young adult (mature) rats.

In some animal models, collagen immunization results in a monophasic, polyarticular, inflammatory arthritis that is mediated by an autoimmune response[14] and that is histopathologically similar to rheumatoid arthritis.[3,9,13] CIA predominantly affects the peripheral appendicular joints and is characterized by intense synovitis and pannus formation, with consequent erosions of cartilage and subchondral bone.[12,15]

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Here we report that juvenile and young adult rats differ in their clinical responses to collagen immunization and that, according to findings from microCT (μCT) and ultrasonography, juvenile and young adult rats differ in their responses to collagen immunization with regard to disease pathology.

Materials and Methods

Animal procedures were approved by the University of Saskatchewan Animal Research Ethics Board (protocol no. 20100140). Juvenile (28 d old, approximately 110 g; $n = 10$) and young adult (84 d old, approximately 375 g; $n = 10$) male Wistar rats (*Rattus norvegicus*; Charles River Laboratories, Sherbrooke, Quebec, Canada) were used. A previous study[4] confirmed that male Wistar rats are sexually mature at postnatal day 70; therefore, we have designated 84-d-old rats as young adults. On arrival at the animal care facility, where the conditions are SPF, the rats were placed 2 per cage on bedding (Care-Fresh, Absorption Corp, Ferndale, WA), kept on a 12:12-h light:dark cycle, and had access to food and water ad libitum. Rats were allowed to acclimate to their surroundings for 1 wk.

Bovine type II collagen (mdBioproducts, St Paul, MN) was dissolved at 4 mg/mL in 0.01 M acetic acid and then diluted to 2 mg/mL with incomplete Freund adjuvant. Eight juvenile (35 d old) and 9 young adult rats (91 d old) were anesthetized with inhaled isoflurane (2% to 4%) and oxygen (1 to 2 L/min). Each of the 17 rats was immunized with bovine type II collagen–adjuvant emulsion by giving 2 intradermal injections (100 μL each) 1.5 cm apart into a shaved area at the base of the tail. Two juvenile and one young adult rats were left untreated and served as naïve controls.

To avoid confounding the clinical scoring and pathologic progression of the disease, no analgesic or antiinflammatory medications were given. Rats were monitored daily for indicators of pain and distress including weight, grooming habits, and the presence of porphyrin around the eyes and nose and on the fur. The paws were scored daily in regard to the degree of inflammation (0, normal; 0.5, erythema and edema in one digit only; 1, erythema and mild edema of the footpad, ankle, or 2 to 5 digits; 2, erythema and moderate edema of 2 joints [footpad, ankle, 2 to 5 digits]; 3, erythema and severe edema of the entire paw; 4, reduced swelling and deformation leading to loss of use of the limb).[13] The maximal daily arthritis score was the sum of the maximal scores for each of the 4 paws. Statistical analyses to evaluate age-related clinical signs were performed by using the nonparametric Mann–Whitney test (Prism 6, GraphPad Software, San Diego, CA).

Two weeks after the clinical onset of arthritis (defined as a maximal daily arthritis score > 0), the CIA rats and the age-matched naïve controls were deeply anesthetized (80 mg/kg; 54 mg/mL; Euthanyl Forte, Bimeda-MTC, Cambridge, Ontario, Canada) and perfused via the aorta with 0.1 PBS followed by 4% paraformaldehyde in 0.1 M PBS. The hindpaws then were removed proximal to the fur line and stored in 4% paraformaldehyde in 0.1 M PBS at 4 °C until imaging.

For µCT image acquisition, each hindpaw (1 naïve juvenile, 4 CIA juvenile, 1 naïve young adult, 4 CIA young adult) was scanned (model 1172, SkyScan, Kontich, Belgium). The hindpaws were rotated through 180° at a rotation step of 0.20°. Each frame was exposed for 0.3 s, and 2-frame averaging was applied to enhance the signal-to-noise ratio. The X-ray energy was standardized at 70 kV and 140 µA, with a nominal resolution of 26.6 µm. A 0.5-mm aluminum filter was used to attenuate low-energy X-rays during the scan. Beam-hardening and ring reduction algorithms (SkyScan) were applied during reconstruction to minimize artifacts. Overall scan time was approximately 1.5 h per paw. Reconstructed scans were imported (Amira 5.4.1, Visage Imaging, Berlin, Germany) for rendering. Surface renders were produced by using standardized thresholds to show only bone. Resampled 2D sections were generated through the tarsus to visualize pathology in the ankle joint.

Ultrasonography was performed by a single experienced sonographer on the same 10 explanted hindpaws as were used for µCT imaging, using a 15- to 7-MHz linear transducer with imaging captured on an ultrasound machine (model iU22, Philips, Andover, MA) to visualize the phalanges, metatarsal-phalangeal joints, metatarsals, tarsal–metatarsal joints, and the tarsus of the paraformaldehyde-fixed hindpaws.

Results

Age-related differences in clinical presentation of CIA. Differences between juvenile and young adult rats with CIA were evident at the time of clinical onset (defined as the first visual signs of erythema and edema). Compared with naïve juvenile controls (Figure 1 A), juvenile rats with arthritis (Figure 1 B) presented with moderate to severe edema predominantly in the metatarsus region and moderate edema extending to the phalanges, which began to resolve by 14 d after arthritis onset (Figure 1 C). In contrast, when compared with young adult naïve rats (Figure 1 D), arthritic rats at the onset of CIA (Figure 1 E) presented with severe edema of the tarsus region, moderate to severe edema of the metatarsus region and moderate edema extending to the phalanges; these changes persisted without resolution at 14 d after arthritis onset (Figure 1 F).

Juvenile rats tended to develop arthritis earlier than did their young adult counterparts; time to onset of CIA after type II collagen injections (mean ± SEM) was 11 ± 1 d for the juvenile rats and 16.3 ± 1.1 d for the young adult rats ($P = 0.0667$, Mann–Whitney test).

Juvenile rats displayed less erythema and edema than did their young adult counterparts; the maximum daily arthritis score for juvenile rats was 4.1 ± 0.2 as compared with 5.4 ± 0.2 for the young adult rats ($P < 0.0001$, Mann–Whitney test). Juvenile rats had fewer days of arthritic paw disuse than did their young adult counterparts (2.8 ± 0.8 d compared with 13.8 ± 0.3 d; $P = 0.0286$, Mann–Whitney test).

Coat appearance and grooming habits. No differences in grooming habits or coat appearance were observed between naïve juvenile and naïve young adult rats. After the onset of arthritis, affected juvenile rats had a mildly rougher coat and mild piloerection, compared with naïve rats. In comparison, affected young adults had substantially rougher coats than did affected juvenile rats. At 14 d after the onset of arthritis, CIA juvenile rats exhibited less over-grooming (as evidenced by redness and scabbing) around the site of immunization than did the CIA young adult rats.

Age-related differences in joint damage in CIA. We used µCT to identify the proliferative and osteolytic lesions of the arthritic hindpaws. Proliferative lesions were defined as reactive formation of new, diffusely distributed and disorganized bone at the periosteal boundary of the bone; osteolytic lesions were defined as erosions of the bone. Compared with naïve juvenile rats (Figure 2 A), juvenile CIA rats showed predominantly proliferative rather than osteolytic pathologic lesions (Figure 2 B). In contrast, compared with naïve young adult (Figure 2 C) and juvenile rats, young adult CIA rats showed both proliferative and osteolytic lesions (Figure 2 D). Juvenile CIA rats exhibited proliferative osteophyte growth (reactive bone) localized over the proximal metatarsus, with negligible extension into the tarsus. Juvenile CIA animals developed osteolytic lesions almost exclusively on the proximal metatarsals (Figure 2 C); in contrast, young adult CIA rats had proliferative osteophytes at the distal interphalangeal joints, over the proximal half of the metatarsus and extending into the tarsus (Figure 2 D).

Examination of 2D image data (Figure 3) demonstrates that both juvenile and young adult CIA rats exhibit qualitative losses in bone density, with young adults being more affected than juvenile rats. Narrowing of joint spaces is evident in the young adult CIA animals. In addition, the production of low-density (reactive) bone is more extensive in the young adult CIA hindpaws than in juvenile CIA hindpaws.

Comparison of naïve juvenile soft-tissue appearance (Figure 4 A) with changes in juvenile rats with CIA (Figure 4 B) by ultrasonography revealed synovial hypertrophy and proliferative low-density bone in the metacarpal phalangeal joints. The young adult rats with CIA (Figure 4 C) demonstrated more florid synovial hypertrophy and proliferative low density bone.

Discussion

Our findings provide evidence of age-related differences in the clinical characteristics and anatomic pathology of the CIA rat model that were revealed by imaging. We observed rapid onset of arthritis in the hindpaws of our rats, which is characteristic of CIA

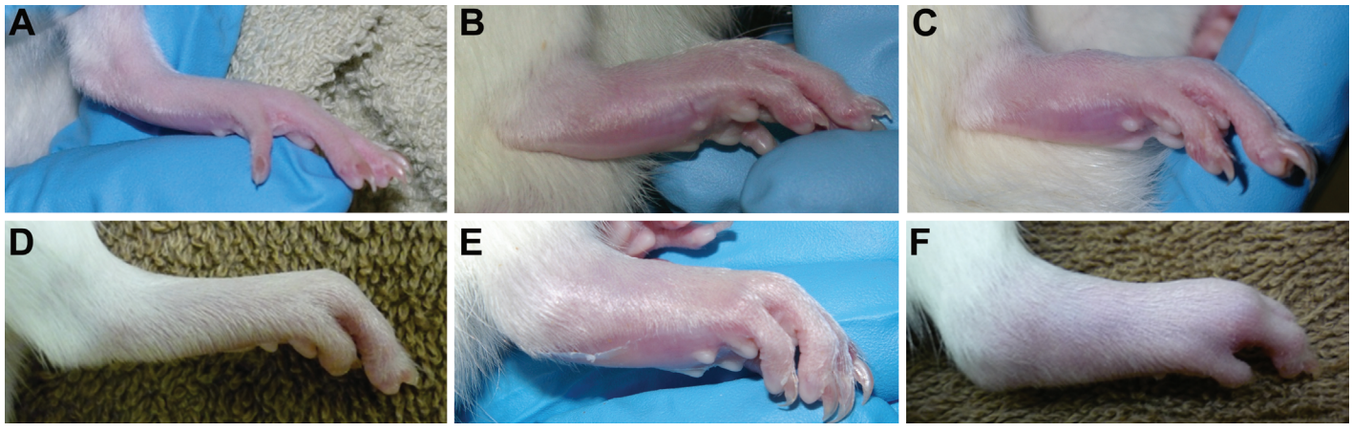

Figure 1. Age-related differences in the clinical presentation of CIA. Photographs of (A through C) juvenile and (D through F) young adult male, Wistar rat right hindpaws. Normal juvenile and young adult paws are depicted in panels A and D, respectively. At the clinical onset of CIA, (B) juvenile rats displayed edema predominantly in the area of the metatarsals extending distally into the phalanges as compared with the (A) naïve juvenile rats. At 14 d after the clinical onset of CIA, (C) the edema in the juvenile rats in the metatarsal and phalanges region was resolving. In contrast, at the clinical onset of CIA, (E) young adult rats displayed extensive edema extending through the entire hindpaw from the tarsus to the distal phalanges when compared with both the (D) naïve young adult rats and (B) juvenile CIA rats at clinical onset. At 14 d after the clinical onset of CIA, (F) the young adult rats continued to display extensive edema throughout the entire hindpaw and the interphalangeal joint regions.

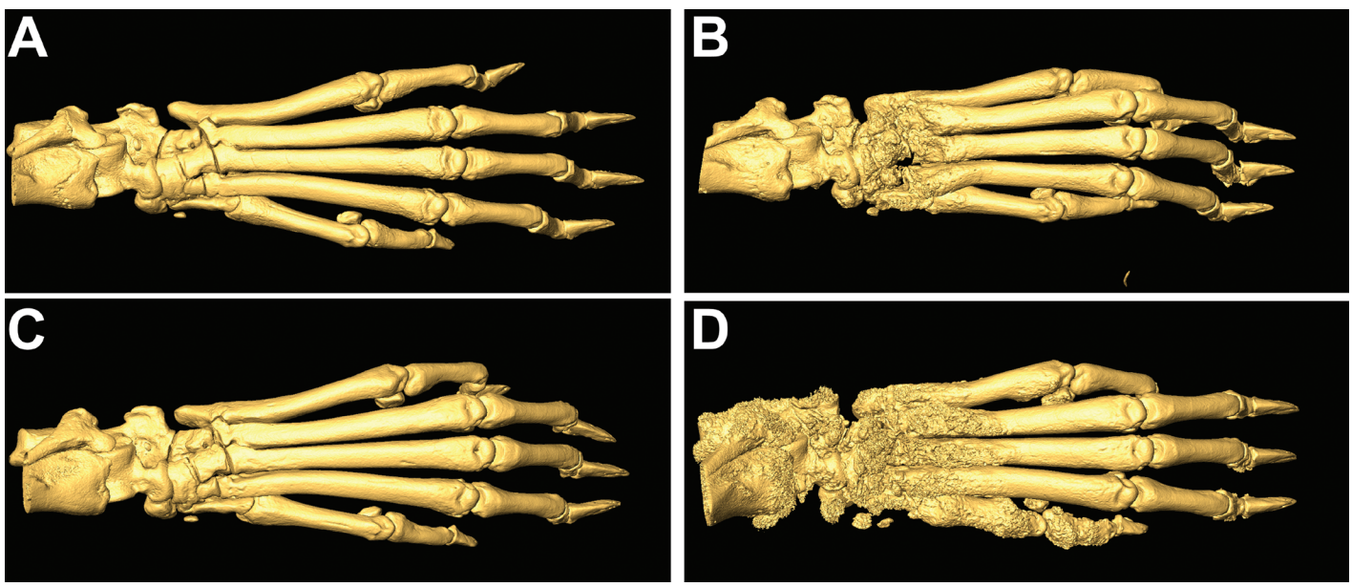

Figure 2. µCT dorsal surface renderings demonstrate qualitative differences in reactive bone between juvenile and young adult rats 14 d after the onset of CIA. Compared with (A) naïve juvenile rats, (B) juveniles rats with CIA developed reactive bone located primarily over the proximal metatarsal regions. In contrast, compared with (C) naïve young adult rats, (D) arthritic young adult CIA rats showed reactive bone that extended over the proximal metatarsus region into the tarsus. In addition, reactive bone was present on the edges of the distal interphalangeal joints in the young adults.

regardless of age, weight, or sex.[1,14,16] We also noted that CIA in young adult rats was characterized by swelling and redness that extended through the ankle and tarsal joints to the distal phalanges, a pattern that has previously been observed in female[14] and male[1] adult rats.

Earlier reports of the time to onset of CIA range from 8 d[16] to 21 d;[2] this variability could relate to variations in immunization protocols including the use of different concentrations of type II collagen, different types of adjuvant, and booster immunization. Our observed times to onset (11 ± 1 d for juvenile rats; 16.3 ± 1.1 d for young adult rats) fall within the previously reported ranges.

Interestingly, our age-related results differed from those of previous reports on experimental arthritis. In contrast to our findings, CIA in female Sprague–Dawley rats (6 wk old and 6 mo old) resulted in similar arthritic index scores between the 2 age groups,[8] and Lewis rats that were 2 to 6 mo of age were more susceptible to adjuvant arthritis and exhibited greater disease severity (significantly higher mean joint score) than did those 18 to 22 mo old.[7] A number of possibilities could explain these differences. First, different strains and sexes of rats (male Wistar compared with female Sprague–Dawley) may respond differently to CIA. Second, unless rats of the same age, strain, and sex are used to generate CIA and

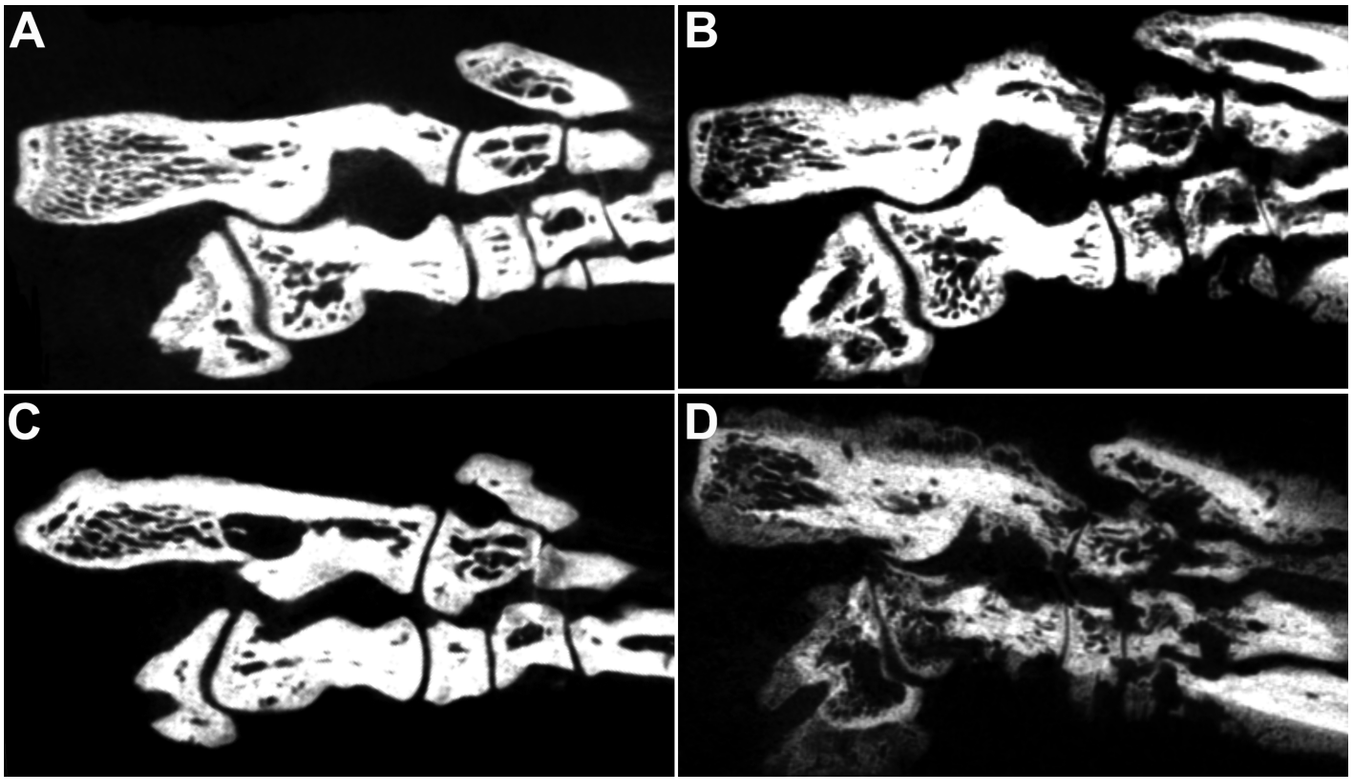

Figure 3. 2D µCT images demonstrate qualitative differences in reactive bone between juvenile and young adult rats 14 d after the onset of CIA. 2D µCT images through the tarsus of (A) naïve juvenile, (B) CIA juvenile, (C) naïve young adult, and (D) CIA young adult rats. Compared with (A, C) their normal counterparts, (B) the juvenile CIA rat shows a qualitative decrease in bone density as well as the development of osteolytic and osteophytic activity in the tarsus.(D) The young adult CIA rat shows more decrease in bone density, more osteolytic activity, and much more proliferation of new bone. Narrowing of the joint spaces is also evident in the young adult.

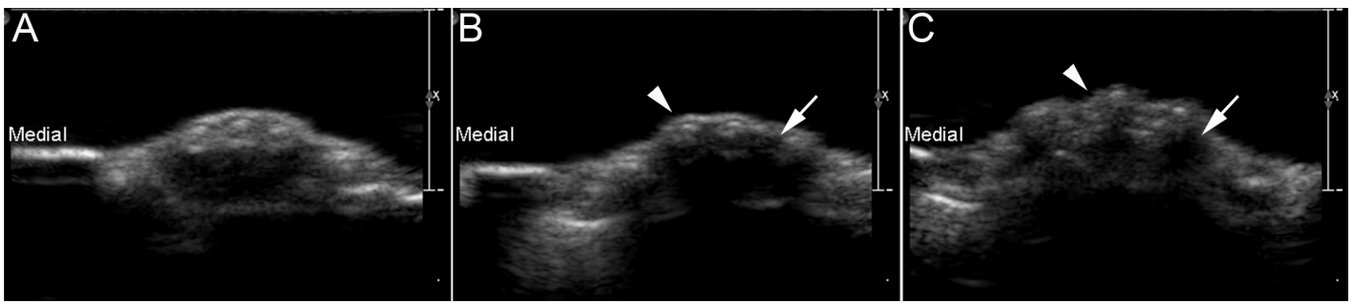

Figure 4. Ultrasonography reveals differences between juvenile and young adult rats with CIA. As compared with (A) naïve juvenile rats, (B) juvenile rats with CIA show soft tissue changes primarily in the region of the phalanges and metatarsals. (C) Young adult rats with CIA showed more severe soft tissue involvement, as evidenced by synovial hypertrophy (white arrowheads), and more bone osteolysis (white arrows).

adjuvant arthritis in the same setting, generalizing between the models may be misleading because the induction method chosen may initiate different clinical responses in the models used. Finally, in one of the cited studies,[7] young rats 2 to 6 mo old were grouped together; this represents a wide age range relative to the lifespan of the animal. Consequently, older rats in the group may have been more susceptible to adjuvant arthritis than were younger ones, but any age-related differences where obscured when the data from younger and older animals were pooled.

The application of µCT and ultrasonography to examine CIA-related changes in affected rat hindpaws allowed us to correlate the presence of inflammation as assessed clinically with anatomic evidence of skeletal and soft tissue pathology. These imaging techniques enable the visualization of proliferative and osteolytic bone lesions, qualitative loss of bone in both cortical bone and trabeculae, and joint-space narrowing, which lesions characterized the differences between juvenile and young adult animals. Ultrasonography is a noninvasive, inexpensive modality that could be used both at the onset of clinical signs and sequentially to describe soft-tissue and bony changes.

Although the time to onset of arthritis was earlier in juvenile rats, their pain, soft-tissue swelling, and extent of destruction was

decreased compared with those of young adults. In addition, in contrast to young adult rats, juvenile rats showed decreased pain and swelling at time of euthanasia. The reasons for the age-related differences we observed are unknown but could relate to a more immature and therefore less reactive immune response in juvenile rats. Increased neuroimmune plasticity may accommodate more rapid immunoinflammatory adaptation in younger animals relative to adults. Support for this hypothesis is based on neuroimmune interactions in young (10 d postnatal) and adult (2 mo old) male Sprague–Dawley rats with peripheral nerve injury, in which macrophages in the adult rats cluster around sensory neuron bodies in the dorsal root ganglion whereas the young nerve-injured rats do not respond differently from young control rats.[17] Applying the CIA model of arthritis in juvenile rodents could help generate new information pertinent to understanding age-dependent mechanisms of arthritis. These new age-related mechanistic insights can inform the development of novel, rationally conceived, biologically based treatment interventions.

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