Chikungunya Virus Infection Alters New Bone Formation Associated with Pre-existing Post-traumatic Osteoarthritis
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Introduction: Chikungunya virus (CHIKV) is a mosquito-borne alphavirus found throughout tropical and subtropical regions. In October of 2013, an outbreak began in the Caribbean and has spread throughout Central and South America, resulting in almost 2 million reported suspected cases. There have been 3,490 reported cases in the U.S. since this outbreak began, including 12 locally acquired cases in Florida in 2014. CHIKV infection results severe polyarthralgia in the 1-3 weeks following infection. Additionally, patients have reported ongoing chronic arthralgia, potentially lasting years after resolution of the initial clinical symptoms. Previous work by the authors has identified novel skeletal and joint lesions associated with CHIKV infection, including periosteal bone proliferation and cartilage necrosis. Reports have suggested that people with pre-existing joint disease, such as osteoarthritis (OA), may be prone to develop more severe and prolonged disease associated with CHIKV infection, though this has not been experimentally examined. Therefore, the goal of this study was to determine if CHIKV infection resulted in more rapid progression of pre-existing OA in an experimental mouse model.

Methods: Animal experiments in this study were approved by the LSU Institutional Animal Care and Use Committee. At 11 weeks of age, female C57Bl/6j mice underwent surgical destabilization of the medial meniscus (DMM) in the right stifle as previously described. Following surgery, mice were allowed to recover for 1 week, then intra-articularly inoculated in the medial aspect of the right stifle joint with either 2X10^4 PFU of virus solution (CHIKV) or a sham inoculation (SHAM), including a group of mice that did not undergo DMM surgery (CTRL). Thusly, there were three treatment groups: DMM+CHIKV (n=8), DMM+SHAM (n=7), and CTRL+CHIKV (n=8). Mice were euthanized 8 weeks post-surgery (7 weeks post-infection), and right hindlimbs were collected and fixed in 10% neutral buffered formalin. Intact stifle joints were scanned with micro-computed tomography (µCT) at 55kV, 0.3-second integration time, and 8µm resolution. Peri-articular osteophyte (OP.V) and subchondral bone (SCB.V) volume were evaluated, along with overall joint morphology. Histopathology of OA severity in the medial joint compartment was evaluated as previously described, including semi-quantitative assessments of articular cartilage structure (ACS, range 1-12) and loss of safranin-O staining (SaO, range 1-12), and histomorphometric analyses of: subchondral bone area (SCB.Ar) and thickness (SCB.Th), periarticular osteophyte area (OP.Ar), and areas of chondrocyte cell death within the articular cartilage (CCD.Ar). Presence or absence and severity of synovitis were assessed using a modified version of an established grading system and these scores were averaged to obtain a total synovitis score (TSS). ANOVA's were performed on µCT and histomorphometric data, Kruskal-Wallis tests were performed on semi-quantitative data. Pearson correlation analyses were performed to correlate size of osteophytes evaluated by both µCT and histomorphometry.

Results: Articular cartilage: No significant difference was identified in ACS scores. A significant difference was identified in SaO scores, where the CTRL+CHIKV had significantly lower SaO scores than both DMM groups (p<0.01). While all three groups demonstrated areas of chondrocyte necrosis, both DMM+CHIKV and DMM+SHAM had significantly higher CCD.Ar than SHAM+CHIKV (p<0.001). Synovitis: A significant increase in TSS was identified in the DMM+CTRL and DMM+CHIKV when compared to the CTRL+CHIKV (p<0.05 for both). Subchondral bone: DMM+CHIKV had significantly higher SCB.Ar and SCB.Th than CTRL+CHIKV (p<0.004 and p<0.001 respectively). SCB.V was significantly higher in the DMM+SHAM compared to both the DMM+CHIKV and CTRL+CHIKV groups (p=0.011 and p<0.001 respectively, Fig. 1). Periarticular osteophytes: A significant positive correlation was identified between OP.V and OP.Ar in both DMM groups only (p=0.022) and when control joints were included (p=0.001). DMM+CHIKV and CTRL+CHIKV had significantly lower OP.Ar than DMM+SHAM (p=0.043 and p<0.001 respectively), and CTRL+CHIKV had significantly lower OP.Ar than the DMM+CHIKV (p<0.001). There were no significant differences identified in OP.V, however subjective evaluation of 3D reconstructions of the stifle joints demonstrated more extensive proliferation of new bone in the DMM+SHAM as compared to the DMM+CHIKV. (Fig. 2)

Discussion: CHIKV infection alone resulted in chondrocyte necrosis, consistent with our previously reported findings. However, concurrent CHIKV did not increase chondrocyte cell death in animals with pre-existing OA, demonstrating that infection does not appear to exacerbate this particular disease manifestation. Our evaluations of synovitis indicate that OA results in more significant synovial alterations than CHIKV infection. This is unexpected, because CHIKV is considered to be an inflammatory arthritis, while OA is degenerative and inflammation is generally considered to be secondary. However, it is possible that inflammation is more severe at this timepoint in OA, whereas the inflammation associated with CHIKV infection may be subsiding at these same timepoints. While µCT analyses did not demonstrate significant differences in osteophyte volumes, histomorphometric measurements that were significantly and positively correlated with µCT data confirmed that CHIKV infection had a significant impact on osteophyte formation. While CHIKV alone does not result in osteophyte development at these timepoints, CHIKV infection in an animal with pre-existing OA resulted in significantly smaller osteophytes compared with OA alone. This suggests that CHIKV may have an inhibitory effect on osteophyte bone formation. SCC measurements indicate that while OA results in expected increases in subchondral bone, concurrent infection with CHIKV diminishes the amount of new SCC formed, which correlates with the osteophyte formation data. With regards to the variation between histomorphometric vs. µCT analyses, histomorphometric measurements only evaluated the medial tibial plateau, whereas µCT analysis involved evaluation of the entire tibial plateau.

Significance: These results indicate that CHIKV infection may have a suppressive effect on bone formation that is associated with compensatory changes in the progression of OA. These results could have significance in clinical monitoring of OA disease progression and treatment choices in individuals with concurrent diseases.


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Figure 1. Subchondral bone volumes as measured by µCT. Means ± SD. * = p<0.05 when compared to DMM+CHIKV and CTRL+CHIKV.

Figure 2. 3D reconstructions of representative stifle joints from (A) DMM+SHAM and (B) DMM+CHIKV, demonstrating displacement of the medial meniscus and abaxial osteophyte formation (arrow heads), as well as more extensive periarticular bone proliferation in the DMM+SHAM group (arrow).