O633
Abstract (oral session)

A rat model for Kingella kingae pathogenesis

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Objectives: Kingella kingae, a Gram negative coccobacillus of the Neisseriaceae family, is a normal inhabitant of the human oropharyngeal flora and an emerging pathogen. The development of new methods for the bacteria isolation and PCR-identification techniques in the 1990s led to the recognition of K. kingae as a leading cause of septic arthritis and osteomyelitis in children younger than two years old. The bacterium is also a cardiovascular pathogen causing infective endocarditis. K. kingae produces a potent protein toxin of the RTX-group, RtxA. Methods: K. kingae septic arthritis isolate PYKK081 was grown on Columbia agar containing 5% sheep blood and 2 µ g/ml vancomycin for 24 h at 37º C, a number of bacteria was identified by colony forming units count. Active RtxA was purifed from PYKK081 cytosolic fraction. The toxin-deficient mutant was created using mariner transposon mutagenesis. Three-week old Sprague-Dawley rats were used in animal models. Mammalian cell lines were obtained from ATCC. Results: K. kingae toxicity: Cohorts of rats were injected intraperitoneally with different doses (from 1x10^9 to 1 x 10^6 cells/animal) of PYKK081, 50% lethal dose (LD50) was 1.3 x 10^8 cells/animal. Septic arthritis model: Right rat knee joints were injected intraarticularly with different doses (from 1x10^5 to 1 x 10^2 cells) of PYKK081. Left knee joint was injected with saline. Thirty one percent of animals injected with 1x10^5 and 1x10^4 bacterial cells demonstrated features of acute inflammation in the infected joint for first 72 h. Histopathological examination of the joint and adjacent bones 7 days after infection revealed the signs of chronic inflammation. RtxA: The secreted RtxA (>1 µ g/ml) had toxic effect on human, rat, rabbit and mouse white blood cells. When tested at high concentrations (>40 µ g/ml), the toxicity of RtxA was also detected on other cell types including human synovial cells and osteoblasts. The toxic effect of the isogenic rtxA mutant on the mammalian cells under the same conditions was not detected. Conclusion: This is the first study toward the development of in vivo model for K. kingae pathogenesis. The bacterium is shown to cause infections in rat offspring. A rat model of knee septic arthritis due to K. kingae is proposed. RtxA primarily affects multiple types of leukocytes suggesting the toxin role in host immune response evasion. RtxA is responsible for the major cytotoxic effect of K. kingae on mammalian cells.