1201. Sarcoidosis Candidate Microbes Identified by Next Generation Sequencing

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Session: P-53. Microbial Pathogenesis

Background. Sarcoidosis is an autoimmune disease characterized by granulomatous lung disease with very prominent mediastinal adenopathy. Acid-fast bacteria, fungi, and viruses have been considered as possible causes of sarcoidosis. We used next-generation or deep sequencing to characterize the microbial content of diseased mediastinal lymph nodes from 10 sarcoidosis patients compared to a set of 10 negative-controls.

Methods. RNA was extracted from fixed paraffinized mediastinal lymph nodes (MLN) from 12 diseased specimens taken from 10 sarcoidosis patients and 2 positive control subjects (TB, MAI), and normal appearing MLN from 10 negative-control subjects (mostly cancer patients). The extracted RNA was sequenced on the Illumina 2500, yielding 125-bp paired-end reads. These reads were aligned to the human genome, human transcriptome, and a nonredundant panmicrobial database. Each experimental sample were compared against the set of 10 negative-controls using the false discovery rate method (q-value). Directed qPCR was performed on all the samples.

Results. 100-153 million read-pairs were obtained from the 24 sequenced samples (12 sarcoidosis, 10 negative-control, 2 positive-control). Among these, 0.01-1.32% of the reads were microbial, with a trend towards fewer microbial reads in the sarcoidosis group compared to controls (means 66K vs. 457K, p=0.09). Mycobacterial sequence was significantly enriched (q< 0.05) in the MAI but not the TB sample compared to the negative-controls. Among the 12 sarcoidosis samples, sequence mappings were significantly enriched (q< 0.05) for the following genera: fungal, Magnaporthe (N=4 samples) and Debaromyces (1); bacteria, Odoribacter (1) and Granulicella (1); and viral, Roseolovirus (6) and Mardivirus (6). Further metagenomic analysis eliminated Magnaporthe as a candidate. qPCR confirmed the presence of Odoribacter in 2 specimens and Debaromyces in 1. Roseolovirus (HHV6) could not be detected by qPCR in any of the samples.

Conclusion. We conclude that sequencing is a feasible method for identifying candidate microbes that might trigger sarcoidosis in human subjects. Further research is required to establish or refute the pathogenicity of these organisms in patients with sarcoidosis.

Disclosures. **All Authors**: No reported disclosures

1202. Subinhibitory Concentrations of Omadacycline Inhibit Staphylococcus aureus Hemolytic Activity in Vitro

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Background. In animal models of *Staphylococcus aureus* infection, α-hemolysin has been shown to be a key virulence factor. Treatment of *S. aureus* with subinhibitory levels of protein synthesis inhibitors can decrease α-hemolysin expression. Omadacycline, a novel aminomethylcycline antibiotic in the tetracycline class of bacterial protein biosynthesis inhibitors, is approved in the United States for treatment of community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI) in adults. This study was performed to determine the durability of inhibition and effect of subinhibitory concentrations of omadacycline on *S. aureus* hemolytic activity.

Methods. All experiments used the methicillin-sensitive *S. aureus* strain Wood 46 (ATCC 10832), a laboratory strain known to secrete high levels of α-hemolysin. Minimum inhibitory concentrations (MICs) of omadacycline and comparator antibiotics (tetracycline, cephalothin, clindamycin, vancomycin, linezolid) were determined. Growth of *S. aureus* with all antibiotics was determined and the percentage of hemolysis assayed. "Washout" experiments were performed with omadacycline only.

Results. *S. aureus* cultures treated with 1/2 or 1/4 the MIC of omadacycline for 4 hours showed hemolysis units/10^8 CFU of 47% and 59% of vehicle-treated cultures, respectively (**Fig. 1A, 1B**). In washout experiments, treatment with as little as 1/4 the MIC of omadacycline for 1 hour decreased the hemolysis units/10^8 CFU by 60% for 4 hours following removal of the drug (**Table 1**).
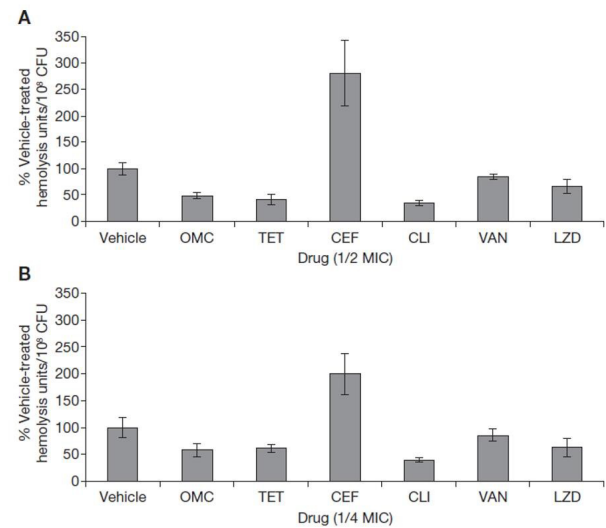
Figure 1

Figure 1. Hemolytic activity of *S. aureus* Wood 46 after 4-hour growth with 1/2 (**A**) and 1/4 (**B**) the MIC of omadacycline (OMC), tetracycline (TET), cephalothin (CEF), clindamycin (CLI), vancomycin (VAN), or linezolid (LZD). Vehicle = 0.0003% DMSO in MH broth. Data represent the mean of 5 cultures; error bars indicate standard deviations. CFU, colony-forming unit; MIC, minimum inhibitory concentration.

Table 1

Table 1. Summary of omadacycline washout study

Fold MIC of omadacycline	% Vehicle-treated hemolysis units/10^8 CFU
0 (vehicle)	100.00 ± 18.59
0.25	39.85 ± 5.03
0.5	55.63 ± 13.40
1.0	15.78 ± 4.66
2.0	21.07 ± 4.29

CFU, colony-forming unit; MIC, minimum inhibitory concentration.
Data indicate mean ± standard deviation of 3 samples per group.

Conclusion. Omadacycline inhibited *S. aureus* hemolytic activity in vitro at subinhibitory concentrations and inhibition was maintained for ≥ 4 hours after removal of extracellular drug (**Fig. 2**). The suppression of virulence factors throughout the approved omadacycline dosing interval, in addition to the in vitro potency of omadacycline, may contribute to the efficacy of omadacycline for ABSSSI and CABP due to virulent *S. aureus*. This finding may apply to other organisms and other virulence factors that require new protein synthesis to establish disease.
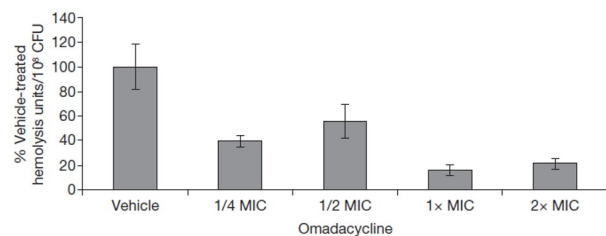
Figure 2

Figure 2. Hemolytic activity of *S. aureus* Wood 46 grown for 1 hour with the indicated concentration of omadacycline followed by 4 hours' growth without drug. Vehicle = 0.0025% DMSO in MH broth. Data represent the mean of 3 cultures; error bars indicate standard deviations.