expression of total eNOS, p-eNOS S1177, total PP2A, and p-PP2A Y307. For activity p-eNOS S1177/total eNOS and p-PP2A Y307/total PP2A ratio was used. A two-way ANOVA was used for statistical analysis. RESULTS/ANTICIPATED RESULTS: Irrespective of the donors’ race, there was no influence of serum treatment or interaction effect in any of the measured proteins of interest. Moreover, compared to CA, HUVECs from AA had lower expression of eNOS irrespective of condition (race p=0.01). Compared to CA, HUVECs from AA tended to have lower expression of p-eNOS S1177 irrespective of condition (race p=0.07). However, there was no racial differences in eNOS activity (p=0.68). There was no racial difference in the expression of PP2A (p=0.35), p-PP2A Y307 (p=0.30), or PP2A activity (p=0.97) in all conditions. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our preliminary results suggest no influence serum constituents from hypertensive donors or race on PP2A or eNOS expression and activity in HUVECs. Future research should consider conducting proteomics profiling to compare NT and HT serum.

**Immune Checkpoint Blockade during Periprosthetic Joint Infection**

Shay Warren, Greg Charville and *Derek Amanatullah
Stanford University

ABSTRACT IMPACT: If immune checkpoint blockade increases bacterial clearance with or without antibiotics in vitro, clinical application would be almost immediate and dramatic creating a seismic shift in the current therapeutic paradigm of periprosthetic joint infection. OBJECTIVES/GOALS: Periprosthetic joint infection (PJI) is a major cause of failure after joint replacement. Currently, the treatment of PJI relies on removing biofilm contaminated implants. Some of the bacteria within biofilm undergo a phenotypic shift becoming small colony variants (SCVs). SCVs induce local immunosuppression through PD-1/L1 signaling. METHODS/STUDY POPULATION: We will infect cultured human macrophages and bone marrow aspirate with stable Staphylococcus aureus SCVs and treat with anti-PD-1 or anti-PD-1 monoclonal antibodies with and without antibiotics (e.g., gentamycin, cefazolin, vancomycin, rifampicin) and assess the residual bacterial viability. We will utilize multiplexed ion beam imaging to quantify PD-1/L1 expression in human tissue from patients with a chronic PJI and compare those to patients undergoing an aseptic revision. Patients with a chronic PJI are likely to have increased expression of PD-1/L1 as their tissue samples are prospectively screened. RESULTS/ANTICIPATED RESULTS: SCVs reduce the phagocytic activity of macrophages and can survive intracellularly. SCVs also induce anti-inflammatory M2-macrophage polarization and recruit a heterogeneous group of immature monocytes and granulocytes called myeloid-derived suppressor cells (MDSC) to the periprosthetic microenvironment. M2-macrophages and MDSCs then produce an immunosuppressive cytokine milieu characterized by increased IL-10 and decreased TNF-α. Clinically isolated SCVs up-regulate the expression of PD-1/L1 and PD-L2 on the surface of macrophages, representing a mechanism by which SCVs induce host immunosuppression and survive immune clearance. Our preliminary data show PD-L1 expression during septic PJI, but not in aseptic revisions. DISCUSSION/SIGNIFICANCE OF FINDINGS: If immune checkpoint blockade is shown to increase bacterial clearance with or without antibiotics, host immunomodulation would represent a novel class of therapeutic adjuvants to assist surgical debridement and antibiotic administration that could be superimposed on existing treatment algorithms to improve PJI related outcomes.