**Nicole Putnam, Ph.D., of Vanderbilt University**[**“The impact of innate immune recognition of Staphylococcus aureus on bone homeostasis and skeletal immunity”**](https://www.niaid.nih.gov/sites/default/files/nicoleputnamapplicationF31.pdf)

**Project Summary/Abstract:**

*Staphylococcus aureus* is a ubiquitous human pathogen, resulting in superficial, invasive, and disseminated infections. One of the most common invasive manifestations of *S. aureus* disease is osteomyelitis, a frequently occurring and debilitating infection of bone. Osteomyelitis triggers dramatic alterations in bone architecture, leading to severe complications such as bone destruction, pathologic fractures, and growth defects. An emerging body of literature suggests that both local and systemic inflammation trigger altered interactions between bone-forming osteoblasts and bone-resorbing osteoclasts to impact bone homeostasis. Skeletal cells are known to express innate pattern recognition receptors (PRRs), but the contribution of innate sensing towards bone homeostasis and antibacterial immunity during *S. aureus* osteomyelitis has not yet been explored. The overarching objective of this proposal is to characterize how innate sensing of bacterial pathogens by skeletal cells triggers alterations in bone physiology. In order to define the impact of skeletal cell PRRs on bone homeostasis, we first focused on the critical signaling adaptor protein, MyD88, which is necessary to transduce signals through toll-like receptors (TLRs) and IL-1 receptors (IL-1R). Our preliminary data demonstrate that MyD88 is necessary to control *S. aureus* replication and dissemination *in vivo* and that osteoclast differentiation can be stimulated by bacterial components in a MyD88-dependent manner *in vitro*. Therefore, the central hypothesis of this proposal is that *S. aureus* modulates osteoclast precursor cell biology and bone remodeling through ligation of osteoclast PRRs and the induction of inflammation. To test this hypothesis, I will use a newly developed murine *S. aureus* osteomyelitis model from our laboratory. This model is advantageous compared to other osteomyelitis models because it allows us to utilize genetically modified animals, high-resolution quantitative imaging analysis, and unique histologic techniques for quantifying perturbations in bone remodeling. Experiments proposed in Aim 1 will investigate the roles of TLR and IL-1R signaling on osteoclast differentiation by monitoring osteoclastogenic signaling cascades, transcription factor activity, expression of mature osteoclast markers, and functionality of osteoclasts formed *in vitro.* Aim 2 will explore how MyD88 signaling in skeletal cells impacts clearance of *S. aureus* and bone remodeling. Collectively, these data will define signaling crosstalk between canonical osteoclast differentiation and innate immune pathways to activate osteoclast differentiation and maturation programs. Additionally, these findings will describe how MyD88 signaling in skeletal cells contributes to immune defenses and affects the kinetics of bone remodeling. This proposed work will have broad implications for how innate skeletal cell sensing contributes to the development of an effective immune response and influences bone homeostasis.

**Nico Contreras, University of Arizona**

[**“The Immunological Consequences of Mouse Cytomegalovirus on Adipose Tissue”**](https://www.niaid.nih.gov/sites/default/files/F31-sample-application_nico_contreras.pdf)

**Project Summary/Abstract:**

Adipose tissue has long been thought to simply be a site of lipid synthesis and energy storage. However, it has become increasingly clear that the inflammatory state of adipose tissue has profound effects on host immunity and metabolism. Recent reports have demonstrated that both viruses and parasites are capable of directly infecting the adipocytes and cellular constituents of adipose tissue. Furthermore, Human Immunodeficiency Virus (HIV) is capable of becoming latent within T cells found in adipose. Cytomegalovirus (CMV), a ubiquitous betaherpesvirus, results in a persistent lifelong infection and the holy grail of CMV research has been to identify sites of latency, but no study has demonstrated the extent to which adipose tissue is infected or harbors latent and persistent virus. CMV has a broad cellular and tissue tropism, and susceptible cells are all represented within the adipose tissue. Thus, it is necessary to investigate the consequences, if any, of CMV infection within adipose. In order to understand the consequence(s) of CMV infection on adipose we will employ the C57BL/6 mouse CMV (mCMV) model of infection. The goal of this proposal is to understand the functional consequences and mechanism of spread during mCMV infection within adipose tissue. The overall hypothesis of this proposal is **that mCMV disseminates to adipose tissue, replicates, establishes latency, leading to an lifelong CD8 T cell response.** We will address the hypothesis and achieve the goals of this proposal by first, determining the cell type(s) that are infected within adipose tissue during infection by qPCR, plaque assay, and flow cytometry. We will also determine if mCMV is capable of becoming reactivated from within adipose tissue. Second, we will determine the kinetic expansion and contraction of mCMV specific CD8 T cells. Investigation into infection of and the role of adipose tissue during an immune response is a new and growing field, thus this work, when completed, will represent a significant advancement in our fundamental base of knowledge regarding mCMV cell tropism and persistence. The findings of this proposal will call for the consideration of adipose tissue in the context of infection, which has far reaching impact on vaccinology, immunology, virology, and endocrinology.

**Samantha Lynne Schwartz, Emory University**

[**“Regulation of 2'-5'-Oligoadenylate Synthetase 1 (OAS1) by dsRNA”**](http://www.niaid.nih.gov/sites/default/files/F31-Sample-Application_Samantha-Schwartz.pdf)

**Project Summary/Abstract:**

The innate immune system is a broad set of critical intracellular and extracellular processes that limit viral infectivity. In order to provide its essential first line of defenses against pathogens, the innate immune system must be able to accurately distinguish “self” from foreign molecules. Misregulation of the innate immune system can cause increased persistence and susceptibility to viral infection and human diseases, such as interferonopathies. The 2’-5’-oligoadenylate synthetase (OAS) family of enzymes are important innate immune sensors of cytosolic double stranded RNA (dsRNA). Attesting to the importance of the OAS/RNase L pathway, viruses have developed ways to evade OAS. Previous structural studies have revealed that dsRNA binding allosterically induces structural changes in OAS1 that reorganize the catalytic site to drive synthesis of 2’-5’-oligoadenylates from ATP. These 2’-5’-oligoadenylate secondary messengers activate a single known target, the latent ribonuclease (RNase L). Active RNase L in turn degrades viral and cellular RNA to halt viral replication. Although X-ray crystal structures have given some insight into how OAS1 is activated by dsRNA, we still understand very little about how specific features of the dsRNA contribute to the level of OAS1 activation. To address which specific features of dsRNA are required for potent OAS1 activation, we designed dsRNA hairpin variants, based on the RNA duplex used in the structural studies. Remarkably, while a single point mutation on one strand resulted in complete loss of OAS1 activity, the equivalent mutation on the opposite strand led to increased OAS1 activity. Despite these stark differences in ability to activate OAS1, both variants appear to bind OAS1 with similar affinity. Given these preliminary findings, I hypothesize that dsRNAs may contain competing OAS1 binding sites with remarkably different capacities to activate the protein in a context dependent manner. However, the molecular signatures defining these sites as activating and non-activating are unknown. The goal of this project is to determine how specific sequences in dsRNA, and their context, control regulation of OAS1 in the following two Specific Aims. Aim 1. To use complementary assays of OAS1 activity in vitro and in human cells to determine the features of dsRNA that lead to potent activation of OAS1. Aim 2. To use biochemical, biophysical, and structural approaches to define the molecular mechanism(s) by which the dsRNA hairpin variants differ in their effects upon OAS1 activation. These experiments will reveal new insights into the regulation of OAS1 by dsRNA. In doing so, I will enhance our understanding of host-pathogen interactions, such as how viruses might circumvent the OAS1/RNase L pathway by masking activating motifs to evade detection. My results will furthermore provide new insights into cellular translational control in the context of infection and potentially strengthen the foundations necessary to design effective treatments for viral infection.