A. Specific Aims

Bacterial pneumonia is a leading burden of disease worldwide and a common cause of acute lung injury. The host immune response to infection must be delicately balanced between mounting an effective defense while preventing excessive lung injury. To coordinate this balance, cytokine expression is dynamically regulated by both transcriptional and post-transcriptional mechanisms of gene expression. Post-transcriptional gene regulation is largely dictated by cis elements that reside within the 3′ untranslated regions (UTRs) of cytokine mRNAs such as AU-rich elements (AREs) and microRNA (miRNA) target sites. As an abundant class of small non-coding RNAs, miRNAs inhibit the translation and stability of target mRNAs. Mature miRNAs are extensively edited and exhibit 3′ terminal sequence variation which is primarily characterized as untemplated adenines or uridines. The enzymes responsible for these modifications and their biological significance are only beginning to be elucidated. We found that a previously uncharacterized protein, Zcchc11, is an active uridyltransferase that binds single stranded miRNAs and catalyzes the transfers of uridine(s) to their 3′ termini. When cells are depleted of Zcchc11, we demonstrated that the protein expression of several pro-inflammatory cytokines and their mRNA stability was inhibited. Furthermore, we deep sequenced miRNAs from control and Zcchc11-depleted cells and found that a subset of mature miRNAs were uridylated in Zcchc11-dependent manner. Excitingly, we saw that although mir-26a represses IL-6 expression during TNF-α stimulation, Zcchc11-dependent uridylation of mir-26a abrogated repressive effects and led to enhanced IL-6 production. This was not the case with mir-365, which maintained its repression of IL-6 despite Zcchc11-dependent uridylation. These initial studies indicated that miRNA 3′ end modifications are indeed biologically significant and support the broad goal of this proposal which is to define the functions of miRNA uridylation during inflammation and determine what roles Zcchc11 plays in this process. Altogether, we will test the **central hypothesis that Zcchc11 uridylates mature miRNAs during pneumonia to regulate cytokine production.** We will pursue this hypothesis by performing the following Specific Aims:

Specific Aim 1. Test the hypothesis that the uridylation of miRNAs by Zcchc11 is stimulated by inflammatory conditions. These experiments will use deep sequencing of small RNA libraries to determine whether the uridylation of mir-26a and other miRNAs is stimulated by cytokines elaborated during pneumonia. Time courses will be characterized in response to treatment with recombinant TNF-α as well as bronchoalveolar lavage fluids from pneumonic mice (which activate NF-κB and STAT3 in cell cultures). Roles of Zcchc11 will be determined using complementary strategies including cell lines in which Zcchc11 is targeted by siRNA and primary cultures in which Zcchc11 is targeted by insertional mutagenesis. The sequence modifications at the 3′ termini of miR-26a and other miRNAs will reveal the effects of inflammation and whether those changes are mediated by Zcchc11.

Specific Aim 2. Test whether sequence elements within uridylated miRNAs and target mRNAs are required for Zcchc11-dependent cytokine expression. We will use IL-6 3′ UTR reporter-based systems to interrogate miRNA and target mRNA interactions in order to identify elements of either RNA molecule that determine whether repression is abrogated by uridylation. The UTR will be mutated to alter the location, surrounding elements, or secondary structure of the target sites in the mRNA to identify which of these factors dictate uridylation effects. Chimeric mutants of mir-26a and mir-365 will be used to assess whether derepression by uridylation is dependent on nucleotides in the miRNA itself. Effects of uridylation on mRNA targets of mir-26a and mir-365 other than IL-6 will be examined to demonstrate whether Zcchc11-mediated uridylation of these miRNAs has consistent effects across multiple transcripts of inflammatory signaling.

Specific Aim 3. Test the hypothesis that Zcchc11 mediates miRNA uridylation, cytokine expression, and innate immunity during pneumonia. Zcchc11-null and wild type C57BL/6 mice will be infected with *E. coli* or *S. pneumoniae* in the lungs. Small RNA libraries from the lungs will demonstrate whether Zcchc11 deficiency alters miRNA uridylation. Cytokines will be measured at RNA and protein levels to determine whether they are regulated by Zcchc11. Effects of Zcchc11 on host defense will be assessed by determining bacterial clearance and neutrophil recruitment, and on lung injury by measuring pulmonary edema, arterial hypoxemia, and loss of lung compliance.