VA Merit Mechanism

• The VA Merit Review Program is considered the VA counterpart of the NIH RO1 funding mechanism.
• Merit Awards are intended to support independent investigators with mature research programs.
• Applying for these grants requires the equivalent of 5/8ths effort.
  – For non-clinician investigators with a doctoral degree, an applicant need not be a current VA employee. However, research must be conducted at the VA Medical Center and applicant must accept a VA-paid appointment of at least 5/8ths time if funded.
• The upper limit on budgets for VA Merit Review proposals has recently been increased to $150,000/year. Since the Principal Investigator's salary (and fringe benefits) are paid from another source, it is not included in this amount.
• The VA Research Service website is an excellent source for more specific information. (http://www.research.va.gov/funding/cdp.cfm)
Mental Health is a Research Priority

• ORD commits a high percentage of funding towards research focused on mental health issues, including anxiety, mood, psychotic, cognitive and behavioral disorders

• Researchers are encouraged to focus on improving mental health care for Veterans
The Path to an R01: Lessons Learned

• Submit - You can’t win if you don’t play
• Ask for and use successful R01 applications as models for your proposal
• Be aware of the review criteria
• Learn about the review process (e.g., serve as a reviewer)
The Path to an R01: Lessons Learned

• The importance of specific aims
  – **Potential problems with specific aims:**
    • Too ambitious, too much work proposed
    • Unfocused aims, unclear goals

• Significance
  – Don’t be afraid to use the word “significance” (e.g., “The significance of the proposed research is.....”)

• Importance of clarity and overall presentation
  – Effective use of figures and diagrams
To define the role of Myosin Va in HIV particle assembly, the motor protein recruited by Rab27a to the secretory lysosome is Myosin Va. Type V myosin mediates vesicle transport, and can interact both with actin filaments and with kinesin, a microtubule-based motor. One model for secretory lysosome transport predicts that recruitment of Myosin Va to vesicles by Rab27a and associated effectors induces movement on microtubules to the cell periphery, then short-range movement on actin filaments. Here we will determine if Myosin Va mediates movement of MVB/secertory lysosomes associated with Gag to the cell periphery.

We will use dominant-negative approaches and inhibitory RNA knockdown to examine the role of Myosin Va in MVB/Secertory lysosomes in HeLa cells and in Hela cells. Expression of the tagged domain of Myosin Va results in a dominant-negative effect in vesicle movement (57). We will express a GFP-tagged Myosin Va tail construct together with Gag-GFP. The tail construct expresses a dominant-negative molecule in this setting, inhibiting transport to the plasma membrane. Movement of Gag-GFP in endosomal compartments to the cell periphery will be monitored by live cell fluorescence imaging, and quantification performed at intervals. We will use a myosin Va tail construct as a control in these experiments. This construct gets access to the cytoskeleton by the Jolliknerin-deduced export out of the recycling endosome compartment (53). Our model predicts that this will interfere with the transport of Gag containing secretory lysosomes to the periphery, while expression of the myosin Va tail construct will. Particle release from cells will then be determined through expression of a Gag-Pro construct with myosin Va tail or myosin Va tail. Quantity of particle release will be performed via p24 antigen capture ELISA.

Role of Rab7, Rab27a, and Myosin Va on Gag trafficking in human macrophages. It remains unknown why particle assembly occurs predominantly in MVBs in macrophages, while particles assemble at the plasma membrane in other cell types. We propose that regulators of vesicular transport may play a role in generating this difference. Here we will examine the role of Rab7, Rab27a, and Myosin Va in the transport of Gag (or particle-containing exosomes) to the plasma membrane of human macrophages. Monocytes are prepared from human PBMCs by magnetic bead immunodepletion of T and B cells, and macrophages prepared by adherence to plastic dishes. The purity of prepared macrophages will be assessed by flow cytometry using anti-CD14 staining. Macrophages will be transfected with VSVG-G-pseudotyped MLV-based retroviral vectors encoding dominant-negative GFP-tagged Rab7, Rab27a, myosin Va tail, or myosin Va (control). Transduced cells will then be infected with VSVG-G pseudotyped NL-4.3 virus. At 12, 24, 36, and 48 hours post-infection, cells will be harvested and fixed for immunostaining. Staining will be performed for detection of gag particles. Staining will be performed for detection of gag particles. The kinetics of viral movement to the plasma membrane will be assayed for each intervention vs. control (non-transduced) cells. The proposed model predicts that Rab7 dominant-negative may result in enhanced movement of the secretory lysosome to the cell surface, while Rab27a or myosin Va will trap more of this compartment within the cell. We will examine the cells by transmission electron microscopy. If the kinetics of viral movement to the plasma membrane are altered, it is possible that the relative amount of MVB versus plasma membrane budding events will be altered. We will score the fields examined for intracellular vs. plasma membrane budding particles to determine if any shift in particle budding has occurred in selected experiments, the identity of the MVB/secertory lysosome compartment. We will also perform staining for Gag and Rab7, Rab27a, and myosin Va in MVBs within infected macrophages.