

## POSTER 31

### UNDERSTANDING THE MECHANISM OF MLV'S RESISTANCE TO MOUSE APOBEC3: MOUSE APOBEC3 PURIFICATION FROM SF9 INSECT CELLS

Silvia Sanchez-Martinez, Jane Mirro, and Alan Rein

HIV Drug Resistance Program, National Cancer Institute-Frederick, Frederick, MD 21702

Mammals have developed several layers of defense against viruses, including an intracellular antiretroviral defense which is also effective against cross-species transmission of retroviruses. An important component of the intracellular antiretroviral defenses are the apobec genes, which are expressed in all mammals, making them very interesting to study as novel antiviral weapons.

In our previous work (Rulli, S.J. et al., 2008; J.Virol. 82: 6566), we compared the antiviral activities of human (hA3G) and murine Apobec3 (mA3) proteins upon Moloney murine leukemia virus (MLV). We found, consistent with previous reports, that human immunodeficiency virus (HIV-1) is able to resist hA3G, due the activity of Vif protein, but is sensitive to mA3, whereas MLV is partially resistant to mA3 but sensitive to hA3G. We observed that both apobec proteins are packaged to similar extents in MLV particles. Unlike hA3G, mA3 in MLV particles failed to cause hypermutation of viral DNA, indicating that its deaminase activity is completely blocked. On the other hand this indicates that mA3 protein inhibits MLV by other mechanisms, independent of its deaminase activity. The nature of this partial resistance, the total resistance to deamination activity, and the mechanism of inactivation of MLV by mA3 are completely unknown.

A detailed biochemical and structural analysis of a purified mA3 protein and a comparison to other zinc-coordinating deaminases can facilitate our understanding of how mA3 binds nucleic acids, recognizes substrates, and forms oligomers.

To address these questions, we decided to create a recombinant baculovirus vector able to express His tag-MBP-mA3 in insect cells. Here we show how we have successfully cloned, transformed, expressed and purified for the first time mA3 from insect cells. We have also been able to partially cleave the fusion protein with TEV protease and recover soluble mA3.

We are focusing our current work on characterizing this purified mA3 biochemically and biophysically and looking for interaction with and inhibition by MLV proteins.