Poster #221

Analysis of Viral Evolution in the Blood Reveals Potential Insight into the Evolution of Macrophage Tropism

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Abstract

Background: Understanding the evolution of entry phenotype of HIV-1 is essential for our understanding of HIV-1 pathogenesis, latency, and disease progression. There is a growing amount of information redefining HIV-1 entry phenotypes. Use of a more precise assay has been developed to define entry phenotype based on the ability of HIV-1 to use CD4 for efficient entry based on density and sensitivity. Infection of macrophages is associated with evolution of the viral Env protein ability to utilize low densities of. We have used this assay to identify infrequent examples of macrophage-tropic (M tropic) virus and partially M tropic in the cerebral spinal fluid and genital tract. However the extent of M tropic virus abundance in other compartments, such as the blood, remains unknown. We set out to determine if M tropic viruses ever reach a point of systemic infection.

Methods: Viral RNA was isolated from blood plasma samples from viremic subjects infected with either subtype B or subtype C HIV-1, and who were late in disease with CD4+ T cell counts of <100 cell/mm3. We performed single genome amplification to isolate individual env gene amplicons, which were cloned and analyzed for CD4 usage using Affinofile cells, a CD4-inducable cell line, in order to determine the viral entry phenotype. Susceptibility of pseudoviruses to soluble CD4 was also evaluated.

Results: To date, no examples of efficient use of low CD4 (M tropic virus) have been found in the blood. However we do occasionally see a level of infection on CD4 low cells that is marginally greater than that of a typical R5 T cell-tropic virus such as JRSCF, which has also been seen in the CSF and genital tract. This new group of viruses showed increased sensitivity to soluble CD4 as well. As expected for late stage subjects, we identified X4 lineages in over 50% of the subjects.

Conclusions: Macrophage tropic viruses were previously considered to be major contributors to infection and disease progression. However, using the direct measurement of the evolutionary step, increased ability to utilize low levels of CD4, as the definition of M tropic viruses leads to the conclusion that these variants are rare and are potentially limited to specific compartments of the body. The appearance of intermediate phenotypes in the blood argues that there may be some evolution toward M tropism taking place in compartments that shed virus into the blood. However, this appears to be restricted to late in disease progression, and is consistent with the virus replicating, at least some of the time, in a low density CD4 expressing cells, such as macrophages.

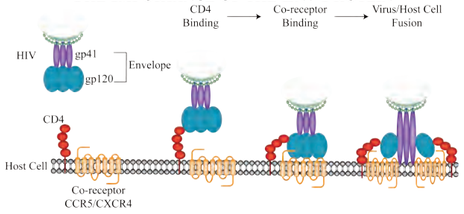
Acknowledgements & Funding Sources

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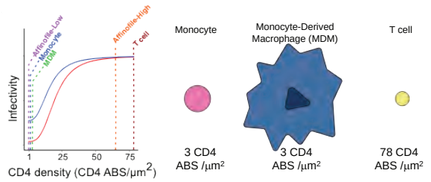
Where Have Macrophage-tropic Viruses Been Found?

The Importance of the Env Protein



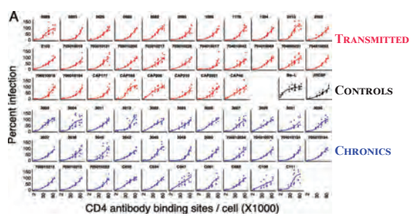
Affinofile Cells Line vs Target Cells In Vivo

Joseph et al. 2013

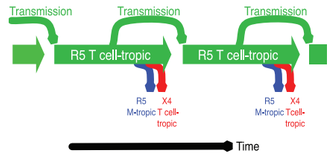


Macrophage-tropic Viruses are Not Transmitted and are Not Easily Found in the Blood in Chronically Infected Subjects with CD4+ T Cells > 200/uL

Ping et al. J. Virol 2013

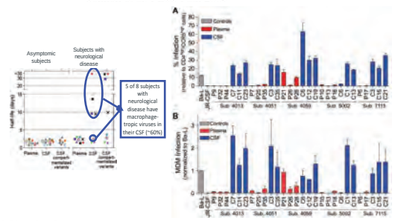


What Does it Mean to be Macrophage-Tropic?



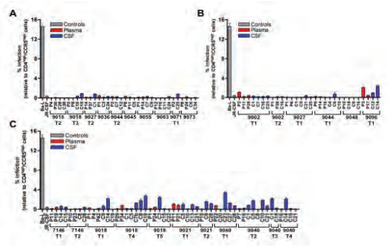
Macrophage-tropic Viruses are Found in the CSF/CNS of Late

Schnell et al. PLoS Path 2009



CSF/CNS Compartmentalized Viruses Early After Infection Can Show a Slightly Improved Ability to Use Low Levels of CD4

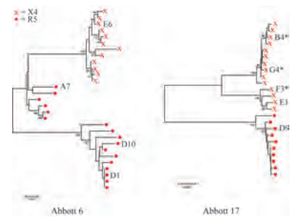
Sturdevant et al. J Virol 2014



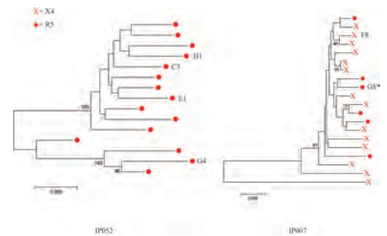
No macrophage-tropic viruses capable of efficiently using low levels of CD4 for entry have been detected in any subject with low levels of CD4+ T cells in the blood. However, there are examples of a low/intermediate phenotype

Representative Trees Illustrating Viral Diversity

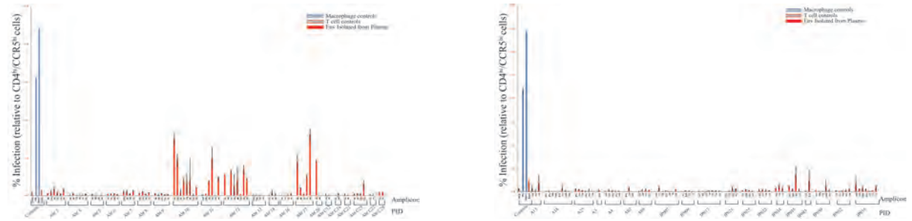
Abbott Samples



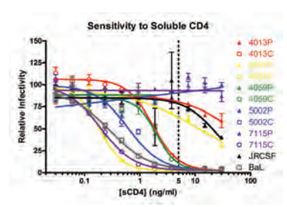
LCD Samples

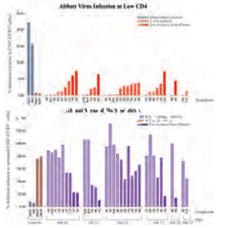


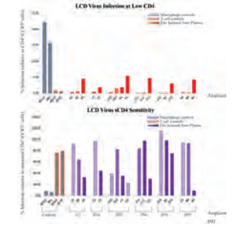
No Virus Isolated From the Blood of Chronic Subjects Can Use Low CD4 (No CCR5 Macrophage Virus in the Blood)



Low/Intermediate Phenotype Viruses are more Susceptible to sCD4







Are Macrophage-tropic Viruses Ever Found in the Blood?

Samples

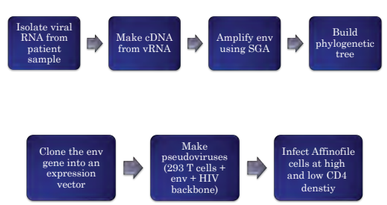
Abbott Subjects

- Ritonavir efficacy study in the 1990's
- Subtype B
- 1090 subjects
  - 543 ritonavir
  - 547 placebo
- For this study
  - 20 placebo subject samples
  - CD4 cells < 100 cells/mm3

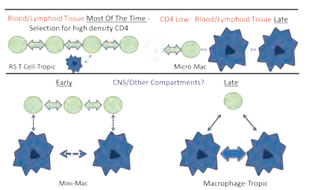
Low CD4 (LCD) Subjects

- Malawi subjects to study the presence of X4 virus
- Subtype C
- For this study
  - 20 placebo subject samples
  - CD4 cells < 100 cells/mm3

Methods



Conclusions



Conclusions: Macrophage-tropic viruses were previously considered to be major contributors to infection and disease progression. Recent availability of a more robust assay allows identification of viruses that have undergone the evolutionary step to utilize low levels of CD4 and allowed us to identify macrophage-tropic viruses as being rare and potentially limited in their evolution to specific compartments of the body. These results argue that there is unlikely to be a significant myeloid component to the latent reservoir, at least comprised of viruses that have evolved to enter cells using low levels of CD4.

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