THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

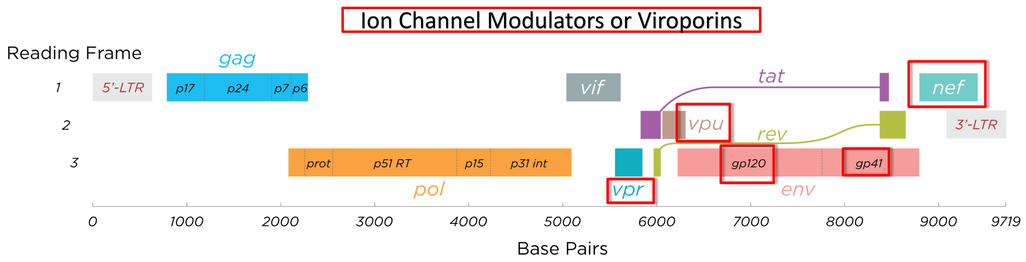
HIV Infected Cells Have Depolarized Membrane Potentials and Increased Intracellular Calcium Levels.

Ryan Goydos[a], Robert L. Furler[a] , and Douglas F. Nixon[a]
[a] The George Washington University, Department of Microbiology, Immunology, & Tropical Medicine

Background:

Ion distribution between the extracellular, cytoplasmic, and organellar spaces creates membrane potentials which drive many of life's processes. Ion channels gate ion entry and exit from the cell to establish a plasma membrane potential that regulates cell size and shape (*1*), migration (*2*), proliferation (*3-5*), and differentiation (*3*, *6-10*). Ion imbalances have been implicated in HIV disease progression. Systemic K^+ alterations have been measured during progressive disease (*11*) and K^+ channel activity has been linked to HIV-associated dementia (*12*). Intracellular K^+ levels are increased during HIV infection and this increase has been associated with cell death (*11-13*).

Several HIV proteins (Vpu, Env, Vpr, and Nef) have been reported to alter host ion channel activity or function as an ion channel. HIV Nef has been shown to inhibit a large-conductance K^+ channel (*13*), inhibit a Ca^{2+}-dependent K^+ channel (*14*), and activate the Ca^{2+} channel IP_3R1 (*15*). HIV Vpu has been shown to depolarize the membrane (*16*) and interfere with the K^+ channel TASK-1 (*17*). Vpu-mediated K^+ channel modulation has been linked to viral budding (*18*, *19*). HIV Env has been shown to interact with the voltage-gated K+ channel BEC1 (*20*). Additionally, like several other viruses, HIV expresses viroporins. These are viral proteins that form ion channels. Ion channel activity has been measured for HIV Vpr (*21*), Vpu (*22-24*), and the cytoplasmic tail of Env/GP41 (*25-29*).



Membrane potential changes during HIV infection may influence cell function and apoptosis. *Hypothesis: HIV depolarizes the plasma membrane and alters intracellular calcium levels. Changing the polarization of the plasma membrane would alter the levels of HIV infection.*

Methods:

Membrane potential and intracellular Ca^{2+} measurements were done on primary human CD4+ T cells from HIV+ individuals. Productively infected cells were identified using a broadly neutralizing anti-Env antibody (PG9) conjugated to AlexaFluor-647. Membrane potential measurements were done by flow cytometry using the membrane potential dye $DiBAC_4$-3. Intracellular Ca^{2+} measurements were done by flow cytometry using the Fluo-4 dye. Ionomycin and PMA were used to show the contrast in intracellular Ca^{2+} levels between infected and uninfected cells. To assess the effects of membrane potential changes on HIV replication, 200μM diazoxide was added to cells during infection of primary PBMC with laboratory HIV-1 isolate NL4-3. To visualize membrane polarization after infection, Jurkat cells were infected with NL-D, a NL4-3 construct containing a dsRed reporter gene (*30*), and incubated with $DiBAC_4$-3. Live cell imaging and analysis was done using a Zeiss spinning disk confocal microscope.

Results:

HIV infected cells consistently had depolarized membrane potentials in both primary cells and cell lines as measured by flow cytometry and visualized by confocal microscopy (Figure 1). This membrane depolarization was accompanied by an increased resting level of intracellular Ca^{2+} in infected cells. Following addition of ionomycin, there was a drastic difference in Ca^{2+} flow between uninfected and HIV-infected cells. In uninfected cells, the addition of ionomycin induced an influx of Ca^{2+} while PMA had little effect. In contrast, both ionomycin and PMA induced a large efflux of Ca^{2+} from HIV infected cells (Figure 2). When cells were cultured with a depolarizing agent, diazoxide, there was an increase in HIV-infected cells (Figure 3).

Conclusions:

Altered Ca^{2+} signaling in infected cells may lead to irregular $CD4^+$ T cell differentiation, function and apoptosis. If HIV-induced effects on membrane potential can be reversed, restoration of cellular function and survival may improve disease progression. Our next step will be to monitor infection in hyperpolarizing conditions.

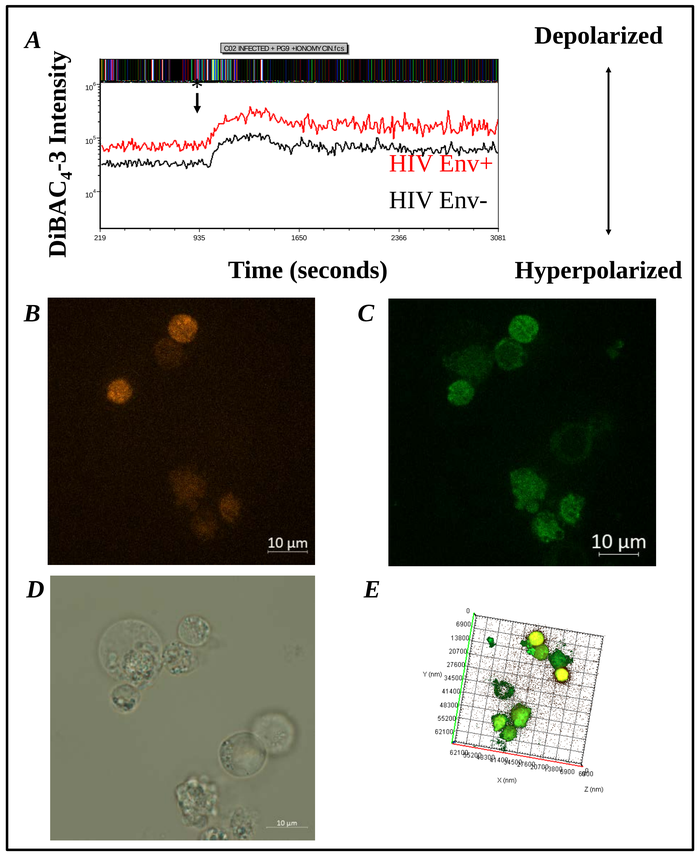


Figure 1: HIV Infected Cells Have Depolarized Plasma Membranes

(***A***) Primary CD4+ T cells from HIV+ participants were gated on HIV infection using the PG9-AF647 Antibody. Using the membrane potential dye $DiBAC_4$-(3), a clear depolarization can be seen in the HIV+ cells (red line) compared to HIV- cells (black line). *Addition of ionomycin. (***B-E***) Jurkat cells were infected with NL-D to visualize membrane potential of infected cells. (**B**) Infected cells contain dsRed. (***C***) Membrane potential is seen using $DiBAC_4$-(3) (***D***) Brightfield image (***E***) 3D reconstruction of cells.

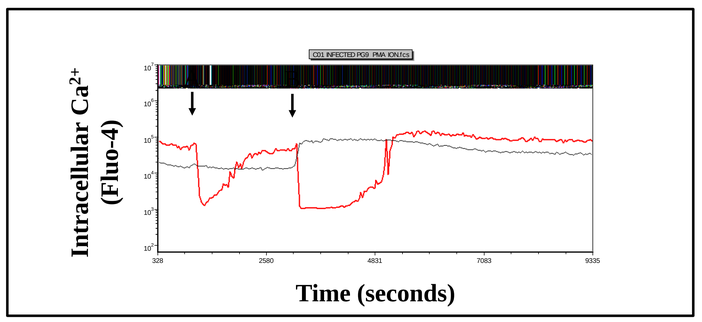


Figure 2: HIV+ Cells Have Altered Intracellular Ca^{2+} Levels.

The levels of intracellular Ca^{2+} levels were measured using Fluo-4 and flow cytometry over several minutes. The black line (HIV-) shows normal Ca^{2+} influx following Ionomycin. The red line (HIV+) shows abnormal efflux of Ca^{2+} following both PMA and Ionomycin stimulation. (A) Addition of PMA (B) Addition of Ionomycin

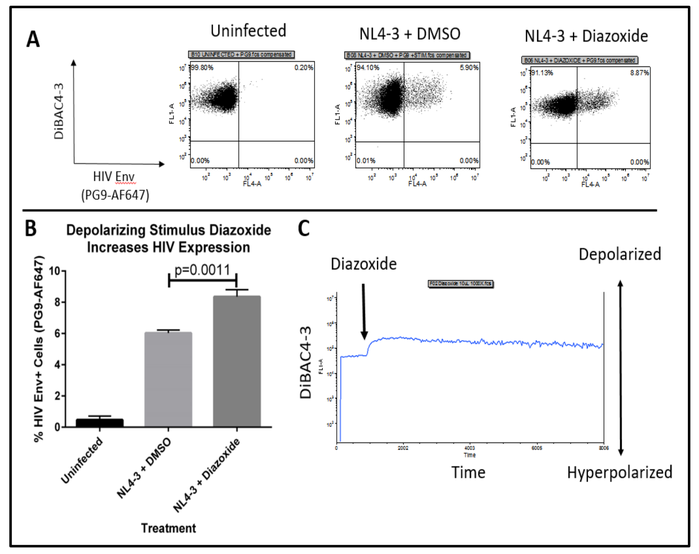


Figure 3: Depolarizing Toxins Increase HIV Replication.

(A) The percentage of HIV+ cells increases from ~6% to ~9% in the presence of depolarizing toxin diazoxide. (B) This increase is significant (p=0.0011). (C) A kinetic plot showing that diazoxide depolarizes the plasma membrane potential.

References:

1. E. K. Hoffmann, I. H. Lambert, S. F. Pedersen, Physiology of cell volume regulation in vertebrates. *Physiological reviews* **89**, 193 (Jan, 2009).
2. A. Schwab, C. Stock, Ion channels and transporters in tumour cell migration and invasion. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **369**, 20130102 (Mar 19, 2014).
3. S. Sundelacruz, M. Levin, D. L. Kaplan, Role of membrane potential in the regulation of cell proliferation and differentiation. *Stem cell reviews* **5**, 231 (Sep, 2009).
4. B. Chernet, M. Levin, Endogenous Voltage Potentials and the Microenvironment: Bioelectric Signals that Reveal, Induce and Normalize Cancer. *Journal of clinical & experimental oncology* **Suppl 1**, (2013).
5. B. T. Chernet, M. Levin, Transmembrane voltage potential of somatic cells controls oncogene-mediated tumorigenesis at long-range. *Oncotarget* **5**, 3287 (May 30, 2014).
6. M. Levin, Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Molecular biology of the cell* **25**, 3835 (Dec 01, 2014).
7. M. Levin, Bioelectromagnetics in morphogenesis. *Bioelectromagnetics* **24**, 295 (Jul, 2003).
8. M. Levin, Endogenous bioelectrical networks store non-genetic patterning information during development and regeneration. *The Journal of physiology* **592**, 2295 (Jun 01, 2014).
9. A. Tseng, M. Levin, Cracking the bioelectric code: Probing endogenous ionic controls of pattern formation. *Communicative & integrative biology* **6**, e22595 (Jan 01, 2013).
10. N. J. Oviedo *et al.*, Long-range neural and gap junction protein-mediated cues control polarity during planarian regeneration. *Developmental biology* **339**, 188 (Mar 01, 2010).
11. C. Caramelo *et al.*, Hyperkalemia in patients infected with the human immunodeficiency virus: involvement of a systemic mechanism. *Kidney international* **56**, 198 (Jul, 1999).
12. J. P. Keblesh, B. C. Reiner, J. Liu, H. Xiong, Pathogenesis of Human Immunodeficiency Virus Type-1 (HIV-1)-Associated Dementia: Role of Voltage-Gated Potassium Channels. *Retrovirology : research and treatment* **2**, 1 (2008).
13. J. J. Kort, T. O. Jalonen, The nef protein of the human immunodeficiency virus type 1 (HIV-1) inhibits a large-conductance potassium channel in human glial cells. *Neuroscience letters* **251**, 1 (Jul 17, 1998).
14. O. Zegarra-Moran *et al.*, HIV-1 nef expression inhibits the activity of a Ca2+-dependent K+ channel involved in the control of the resting potential in CEM lymphocytes. *Journal of immunology (Baltimore, Md. : 1950)* **162**, 5359 (May 1, 1999).
15. A. Manninen, K. Saksela, HIV-1 Nef Interacts with Inositol Trisphosphate Receptor to Activate Calcium Signaling in T Cells. *The Journal of Experimental Medicine* **195**, 1023 (2002).
16. K. Hsu, J. Han, K. Shinlapawittayatorn, I. Deschenes, E. Marban, Membrane potential depolarization as a triggering mechanism for Vpu-mediated HIV-1 release. *Biophysical journal* **99**, 1718 (Sep 22, 2010).
17. K. Hsu, J. Seharaseyon, P. Dong, S. Bour, E. Marban, Mutual functional destruction of HIV-1 Vpu and host TASK-1 channel. *Molecular cell* **14**, 259 (Apr 23, 2004).
18. T. G. Voss, P. J. Gatti, C. D. Fermin, R. F. Garry, Reduction of human immunodeficiency virus production and cytopathic effects by inhibitors of the Na+/K+/2Cl- cotransporter. *Virology* **219**, 291 (May 1, 1996).
19. D. T. Huang, N. Chi, S. C. Chen, T. Y. Lee, K. Hsu, Background K(2P) channels KCNK3/9/15 limit the budding of cell membrane-derived vesicles. *Cell biochemistry and biophysics* **61**, 585 (Dec, 2011).
20. M. Herrmann *et al.*, Interaction of human immunodeficiency virus gp120 with the voltage-gated potassium channel BEC1. *FEBS letters* **584**, 3513 (Aug 20, 2010).
21. S. C. Piller, G. D. Ewart, D. A. Jans, P. W. Gage, G. B. Cox, The amino-terminal region of Vpr from human immunodeficiency virus type 1 forms ion channels and kills neurons. *Journal of virology* **73**, 4230 (May, 1999).
22. U. Schubert *et al.*, Identification of an ion channel activity of the Vpu transmembrane domain and its involvement in the regulation of virus release from HIV-1-infected cells. *FEBS letters* **398**, 12 (Nov 25, 1996).
23. K. Strebel, HIV-1 Vpu - an ion channel in search of a job. *Biochimica et biophysica acta* **1838**, 1074 (Apr, 2014).
24. T. Mehnert *et al.*, Biophysical characterization of Vpu from HIV-1 suggests a channel-pore dualism. *Proteins* **70**, 1488 (Mar, 2008).
25. T. S. Postler, R. C. Desrosiers, The tale of the long tail: the cytoplasmic domain of HIV-1 gp41. *Journal of virology* **87**, 2 (Jan, 2013).
26. A. M. Comardelle *et al.*, A synthetic peptide corresponding to the carboxy terminus of human immunodeficiency virus type 1 transmembrane glycoprotein induces alterations in the ionic permeability of Xenopus laevis oocytes. *AIDS research and human retroviruses* **13**, 1525 (Nov 20, 1997).
27. J. Arroyo, M. Boceta, M. E. Gonzalez, M. Michel, L. Carrasco, Membrane permeabilization by different regions of the human immunodeficiency virus type 1 transmembrane glycoprotein gp41. *Journal of virology* **69**, 4095 (Jul, 1995).
28. L. Chernomordik, A. N. Chanturiya, E. Suss-Toby, E. Nora, J. Zimmerberg, An amphipathic peptide from the C-terminal region of the human immunodeficiency virus envelope glycoprotein causes pore formation in membranes. *Journal of virology* **68**, 7115 (Nov, 1994).
29. J. M. Costin, J. M. Rausch, R. F. Garry, W. C. Wimley, Viroporin potential of the lentivirus lytic peptide (LLP) domains of the HIV-1 gp41 protein. *Virology journal* **4**, 123 (Nov 20, 2007).
30. K. Terahara *et al.*, Fluorescent Reporter Signals, EGFP, and DsRed, Encoded in HIV-1 Facilitate the Detection of Productively Infected Cells and Cell-Associated Viral Replication Levels. *Frontiers in microbiology* **2**, 280 (2011).

Acknowledgements: This work was done with the generous support of Dr. Douglas F. Nixon, Dr. Michael Levin, and a DC CFAR Microgrant.

DC CFAR District of Columbia Center for AIDS Research