

## I. INTRODUCTION

### Major research areas

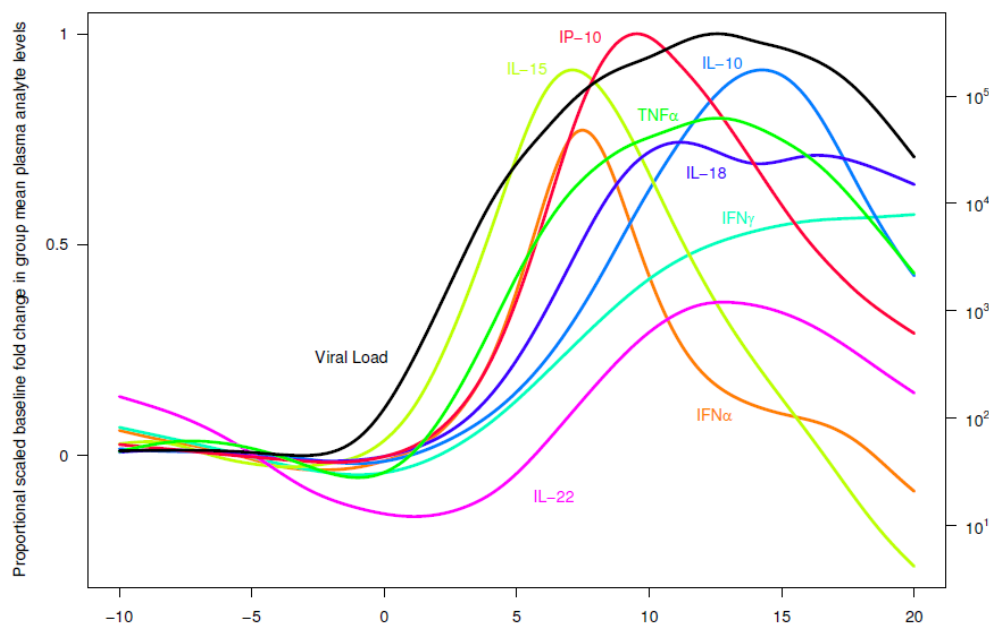
CD4+ CTL and HIV  
 Primary HIV infection  
 CD4+ T cell antigenic activation  
 West Nile virus (WNV)  
 HTLV Immunology  
 Influenza viremia in blood donors

## II. PROGRAM SUMMARY

### Primary HIV infection

Early HIV infection has formed one of the key research areas for the Norris lab since arriving in San Francisco in 2004. The first project has focused on providing detuned HIV antibody testing in support of cohorts such as the Options cohort in San Francisco. The detuned HIV test was developed by Dr. Michael Busch and colleagues and is used to help identify HIV positive subjects within six month window of infection. The assay is performed in the core immunology laboratory supervised by Dr. Norris and has formed the basis for a number of collaborations, including a publication detailing the waning of antibody responses in subjects treated with highly active antiretroviral therapy (1) and on the early detection of p24 antigen (2). It is anticipated that detuned HIV testing will continue over the next number of years. A significant challenge in the field is that the currently licensed assay used for detuned HIV testing has been discontinued. Dr. Sheila Keating has been recruited to take over the validation and supervision of the detuned HIV assay work as the new director of the Core Immunology Lab.

The second focus of research activity in early HIV has been in defining the cytokine profile associated with acute HIV infection. We originally worked with plasma donor panels to track cytokine measurements spanning time points prior to infection through the acute period of viremia and seroconversion. Initial studies performed using high-sensitivity ELISA kits revealed early elevations in IL-10, TNF- $\alpha$ , and IFN- $\gamma$  in primary HIV (3). Since the initial publication, high-sensitivity multiplex cytokine testing has become available on the Luminex platform using bead-coupled reagents collected using flow-cell technology. These assays have exponentially expanded the amount of data that can be collected from a single plasma or serum sample. As part of the Center for HIV/AIDS Vaccine Immunology (CHAVI), we have generated a much more comprehensive picture of the evolution of cytokine responses in early HIV infection (Fig. 1).



**Figure 1. Proportion of subjects with cytokine elevations in acute HIV infection.** Early innate immune mediators such as IP-10, IFN- $\alpha$ , and TNF- $\alpha$  arose earliest and in the highest percentage of subjects with acute HIV infection. Downstream adaptive cytokines were elevated later and in a lower proportion of subjects.

The HIV positive plasma donors have been compared to those with HBV and HCV infection, revealing that HIV causes a robust cytokine response that is much higher in breadth and magnitude than other chronic viral infections (data not shown).

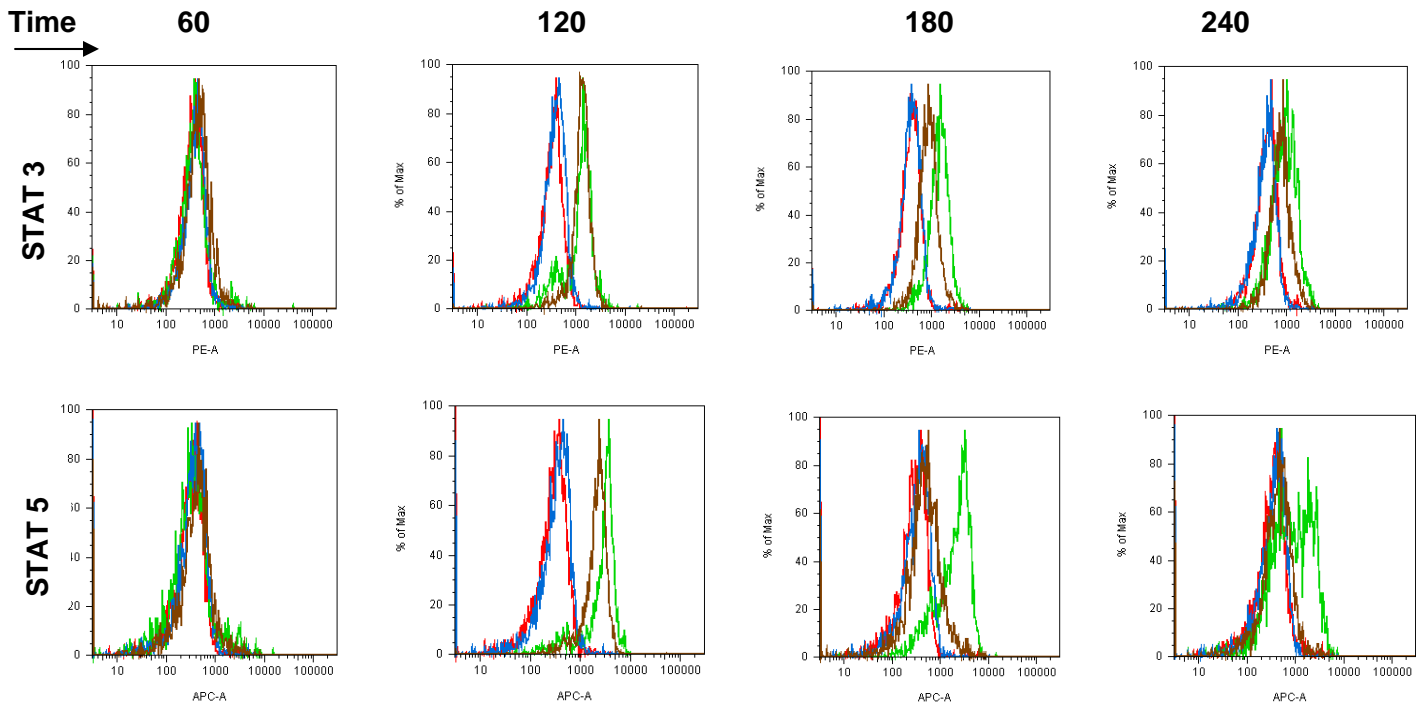
#### Effector CD4+ T cells and HIV

There are two projects related to effector CD4+ T cells. The first, performed by Dr. Rachel Owen, is to quantify the frequency of *ex vivo* HIV-specific CD4+ T cells and correlate the frequency with the ability of these cells to kill cells expressing HIV proteins. These experiments will be performed in patients with and without control of HIV replication, with the hypothesis being that CD4+ CTL activity will be associated with control of HIV replication. The initial screening phase of the experiment has been completed in collaboration with Drs. Steven Deeks and Brinda Emu at UCSF, identifying a panel of rare individuals with high frequency HIV-specific CD4+ T cell responses. Dr. Owen's project has been significantly impeded by the finding that HIV-specific CD4+ T cell responses wane after long-term cryopreservation, greatly complicating study design. Dr. Owen's data suggest that HIV-specific CD4+ T cells undergo increased rates of apoptosis upon stimulation after long-term storage, and these findings formed the basis for her first publication (4).

The second CD4+ CTL project, performed by Dr. Moraima Pagan, focuses on how HIV escapes from CD4+ T cell responses. In early work we showed that HIV-specific CD4+ T cells can lyse HIV-infected target CD4+ T cells and can suppress viral replication in an MHC-restricted and cell contact dependent manner (5). To test whether this suppression will result in viral escape, both X4- and R5-tropic HIV will be replicated in the presence of HIV-specific CD4+ T cell clones. Dr. Pagan has demonstrated that a number of HIV-specific clones can drive the evolution of HIV, with mutations arising within the T cell epitopes recognized by a series of HIV-specific CD4+ T cell clones. Furthermore, she has demonstrated that the mutated HIV sequence codes for peptides not recognized by the effector CD4+ T cells.

#### CD4+ T cell antagonism and HIV

We developed a panel of five HIV-specific CD4+ T cell clones derived from subjects with antiretroviral treated acute HIV or long-term nonprogressive infection. Challenging this panel of clones with commonly occurring viral variant sequence peptides revealed that most variants of HIV are poorly recognized (6). We subsequently defined a system illustrating the ability of HIV peptides to antagonize the response of HIV-specific CD4+ T cells to cognate antigen. The definition of the peptide length-dependent antagonism formed the basis for crystallization experiments performed by our collaborator Dr. Lawrence Stern at the University of Massachusetts. Using x-ray crystallography he demonstrated that the C-terminus of a very long (16 amino acids) minimum epitope bends back towards the MHC-T cell receptor (TCR) complex to interact with the TCR (7). In 2006 we published a manuscript detailing the phenomenon of antagonism and providing molecular detail of the antagonist versus agonist peptide in their interaction with the MHC molecule (8). We showed that the antagonist peptide-MHC could still bind to the T cell receptor, albeit with less affinity than the agonist peptide. We also generated exciting preliminary data using microarrays and intracellular staining for the phosphorylation state of T cell activating proteins showing that the antagonist peptide caused an interruption of the IL-2 signaling pathway in CD4+ T cells (Fig. 2). These findings have implications for basic T cell immunology, as well as HIV vaccine development. Defining how T cells recognize cognate antigen has been a main thrust of the Norris lab (9-12).

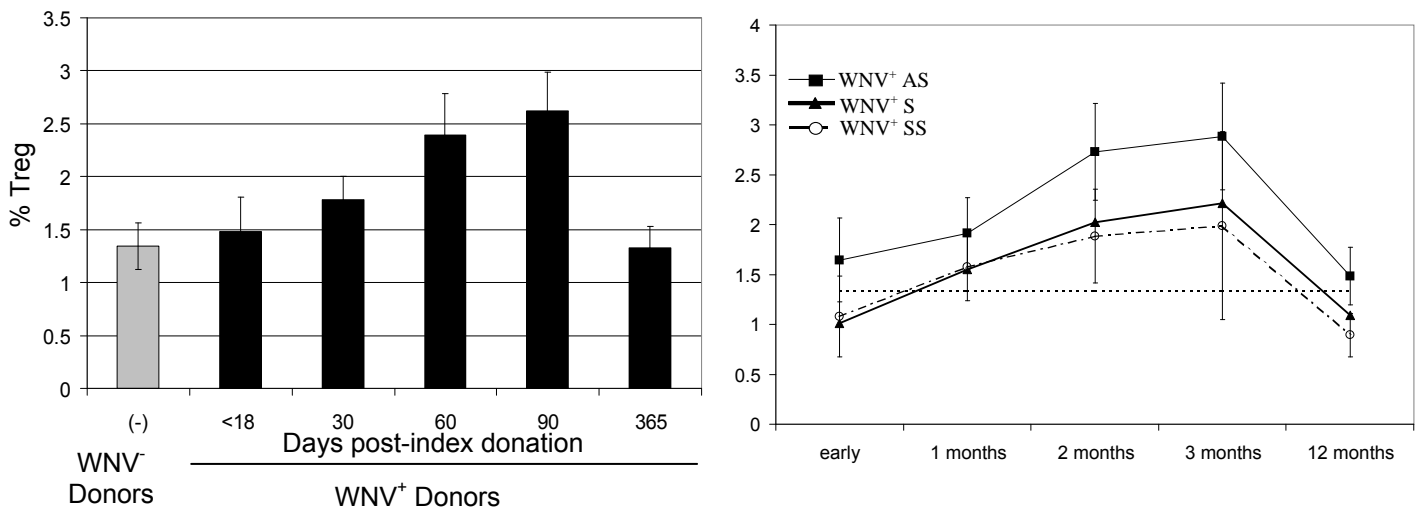


**Figure 2. Antagonism prevents sustained T cell activation.** Phosphorylation of STAT3 and STAT5 was detected 120 minutes after stimulation with agonist alone or agonist plus antagonist. Sustained stimulation of STAT3 and particularly STAT5 was only observed after stimulation with agonist alone. At least 10,000 events were collected per condition and analysis was gated on CD4+ cells.

■ No stimulation  
■ Agonist peptide alone  
■ Antagonist peptide alone  
■ Agonist + antagonist peptides

### West Nile virus (WNV)

Dr. Marion Lanteri's project has been to define the immune response associated with acute WNV infection. Overlapping pools of peptides spanning the whole WNV proteome have been used to determine which areas of the virus are recognized by T cells in infected subjects. In addition, the phenotype and HLA restriction of responding T cells has been characterized for those subjects with high-level responses. These findings formed the basis for a manuscript under review at the Journal of Infectious Diseases (13). Finally, the frequency of regulatory T cell responses have been measured longitudinally through the acute period of infection (Fig. 3A). We have shown that the Treg cell markers are consistent with classic Tregs and that cells sorted based on these markers will suppress proliferation of target cells (data not shown). The frequency of these responses rises significantly by one month after infection. By separating subjects based on whether or not they developed symptoms of WNV infection, it can be seen that symptomatic subjects have lower levels of Tregs early in infection (Fig 3B). Most remarkably, Treg levels at one year, when Treg frequencies had returned to normal control levels, were still significantly lower in patients who had suffered symptomatic WNV infection. These results imply that "Treg tone" may predict how people will respond to acute viral infection.



**Figure 3. Longitudinal Treg frequency in symptomatic vs. asymptomatic subjects.**

**A.** Average Treg frequencies of 29 WNV infected subjects peaked at 3 months. Treg were measured as percent of viable lymphocytes, excluding EMA, CD14, CD16, CD19 positive cells. Treg were defined as CD4+CD25+CTLA4+CD127<sup>-</sup>. Control WNV negative subject samples were run in parallel with each assay.

**B.** Treg levels were statistically significantly higher using the generalized estimating equation for asymptomatic patients (AS) compared to symptomatic (S) or severely symptomatic (SS) patients.

### HTLV Immunology

The goal of this project is to correlate lymphocyte activation with outcome in a cohort of subjects prospectively followed for over ten years. Spontaneous lymphocyte proliferation is a feature of HTLV infection. Using banked frozen specimens, we will measure spontaneous lymphocyte proliferation in subjects who have developed complications of HTLV infection such as HTLV-associated myelopathy or T cell leukemia. We have purified anti-Tax antibody from a hybridoma and have conjugated the purified antibody to a fluorochrome to monitor proportion of spontaneously proliferating lymphocytes is expressing Tax. We are currently enumerating which subsets of lymphocytes spontaneously proliferate in subjects with HTLV-I infection and are correlating these findings with intracellular Tax expression, allowing insight to the pathogenesis of HTLV infection.

### Influenza viremia in blood donors

Influenza remains a major cause of morbidity and mortality in the U.S. and worldwide. The threat of pandemic influenza A has recently gained prominent attention with human cases of highly pathogenic avian influenza A detected in China, Southeast Asia, and more recently in Turkey. The threat that influenza poses to the blood supply remains largely unexplored. Viremia during the symptomatic phase of influenza is rare (14), and the frequency of viremia during presymptomatic or asymptomatic infection is unknown. If influenza infection resulted in a viremic phase prior to the onset of symptoms, it would have implications for blood safety. Due to their underlying health problems, blood product recipients would be expected to suffer increased morbidity and mortality secondary to influenza A infection compared to healthy donors. If influenza were transmissible by transfusion, testing of the blood supply could become particularly important during pandemic outbreak of highly pathogenic influenza A. We are currently worked with commercial and government suppliers of influenza testing kits to measure the relative sensitivity and lower limit of detection of nucleic acid amplification-based tests to detect the presence of influenza in blood. Testing of samples from a large NIH repository containing samples collected during periods of influenza outbreak in the community revealed no cases of viremia. If blood donors harbor influenza viremia during the pre-symptomatic phase of infection we would expect to see increased rates of influenza viremia during periods of disease activity. Initial testing of approximately 50 samples collected through UBS centers did not reveal any with viremia. In 2009 a panel of specimens will be collected from the American Red Cross, which will complete the panel of specimens for this study.

### Anti-HLA antibody prevalence in blood donors

As part of the Core Immunology laboratory the Norris lab measured the presence of anti-HLA antibodies in 9,000 blood donor specimens. Anti-HLA antibodies may be implicated in the pathogenesis of transfusion-related acute lung injury (TRALI). The project required assay optimization, data review, and creation of an expert review panel to interpret the complicated anti-HLA antibody results. While mainly a core lab function, a methods manuscript has been published and a blood bank related manuscripts detailing the prevalence of anti-HLA antibodies in various donor populations and the implications for the safety and availability of the blood supply is under review at JAMA.

#### Transfusion-associated microchimerism

Microchimerism is defined as the persistence of a minor population of allogeneic cells within a host recipient. This can occur after events such as pregnancy, with persistence of fetal cells in the maternal circulation, and overt chimerism occurs after bone marrow transplant. Microchimerism has also been shown to occur in transfusion recipients who receive blood in the post-trauma phase, and prolonged persistence of chimeric cells can occur without ongoing exogenous immunosuppression (15). It is remarkable that the transient immunosuppression associated with trauma will allow persistence of allogeneic cells for up to at least one year post-transfusion. The Norris lab is tasked with exploring how allogeneic donor cells persist in trauma patients who develop microchimerism post-transfusion. The studies will focus on immune function at and shortly after the time of transfusion, as well as on longitudinal samples obtained from subjects who have persistent microchimerism and those who clear the allogeneic cells. We hypothesize that those subjects who develop microchimerism will have more profoundly disrupted immune systems at the time of trauma and will be better matched to the donors implicated in microchimerism than to those donors whose cells do not persist. Many of the techniques to be used in the microchimerism studies, such as multiplex cytokine testing and Treg quantification, have been developed in prior studies of immune responses to viral infections. The microchimerism studies will hopefully provide significant advances in our understanding of immune tolerance and will provide substantial funding to the Norris lab for several years. Dr. Rachael Jackman has been recruited to work on this project and is well on her way to her first manuscript on the topic, detailing early cytokine profiles in transfused trauma patients with or without microchimerism.

### **III. OTHER SIGNIFICANT ACTIVITIES**

Associate Director, UCSF-GIVI Center for AIDS Research

Member, Academic Promotions Committee, Department of Laboratory Medicine

Mentor, UCSF-GIVI CFAR Mentoring Program

Infectious Diseases Consult Attending, SFGH

Chair, UCSF Resource Allocation Program, Basic HIV/AIDS, Infectious Diseases, Global Health Review Committee

#### **Recent publications**

1. Lanteri MC, Heitman JW, Owen RE, Busch TA, Gefter N, Kiely N, Kamel HT, Tobler LH, Busch MP, and **Norris PJ**. Comprehensive analysis of West Nile virus T cell responses in human infection. *J Infect Dis* May 1;197(9):1296-306, 2008.
2. Ndhlovu LC, Chapman JM, Jha AR, Snyder-Cappione JE, Pagan M, Leal FE, Boland BS, **Norris PJ**, Rosenberg MG, Nixon DF. Suppression of HIV-1 plasma viral load below detection preserves IL-17 producing T cells in HIV-1 infection. *AIDS* May 11;22(8):990-2, 2008.
3. Tobler LH, Cameron MJ, Lanteri MC, Prince HE, Danesh A, Persad D, Lanciotti RS, **Norris PJ**, Kelvin DJ, Busch MP. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. *J Infect Dis* Oct 1;198(7):979-983 (2008).
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5. Beal AM, Anikeeva N, Varma R, Cameron TO, **Norris PJ**, Dustin ML, Sykulev Y. PKC $\theta$  regulates stability of the peripheral adhesion ring junction and contributes to the sensitivity of target cell lysis by CTL. *J Immunol* Oct 1;181(7):4815-24(2008).
6. **Norris PJ**, Lee JH, Carrick D, Gottschall JL, Lebedeva M, De Castro R, Kleinman SH, Busch MP. Long-term positivity for anti-HLA antibodies and comparison of detection using serum vs. plasma. *Transfusion* (in press).
7. Hatano H, Delwart EL, **Norris PJ**, Lee T-H, Dunn-Williams J, Hunt PW, Hoh R, Stramer SL, Linnen JM, McCune JM, Martin JN, Busch MP, Deeks SG. Evidence for persistent low-level viremia in individuals who control HIV in the absence of antiretroviral therapy *J Virol* 83(1):329-335 (2009).

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3. Norris, P. J., B. L. Pappalardo, B. Custer, G. Spotts, F. M. Hecht, and M. P. Busch. 2006. Elevations in IL-10, TNF-alpha, and IFN-gamma from the earliest point of HIV Type 1 infection. *AIDS Res Hum Retroviruses* 22:757-762.
4. Owen, R. E., E. Sinclair, B. Emu, J. W. Heitman, D. F. Hirschorn, C. L. Epling, Q. X. Tan, B. Custer, J. M. Harris, M. A. Jacobson, J. M. McCune, J. N. Martin, F. M. Hecht, S. G. Deeks, and P. J. Norris. 2007. Loss of T cell responses following long-term cryopreservation. *J Immunol Methods*.
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15. Reed, W., T. H. Lee, P. J. Norris, G. H. Utter, and M. P. Busch. 2007. Transfusion-associated microchimerism: a new complication of blood transfusions in severely injured patients. *Seminars in hematology* 44:24-31.