Project: Understanding poor vaccine responses: transcriptomics of vaccine failure

Applicant: Kathleen Miller, Department of Microbiology & Immunology

Supervisor: Dr. Lisa Barrett, Dalhousie University and Nova Scotia Health Authority

BACKGROUND: Immune senescence is increased in the aging population and is associated with increased rates of infection[1] and poor vaccine response[2]. The development of better strategies for reducing age-related infection and improving responses to vaccination is restricted by our lack of knowledge of how and why the human immune system becomes less effective with age. As traditional chronologic aging studies take a great deal of time, we utilize chronic viral infection as an accelerated model of aging. Similar to the aging population, individuals with chronic HIV infection display an immunosenescent phenotype[3] and often have poor antibody responses to influenza vaccination[4]. Clinically, HIV infected individuals get influenza frequently, despite vaccination[5]. Understanding the elements of immunosenescence that lead to poor vaccine response can aid in the development of better vaccination strategies.

Transcriptomics provide a high throughput analysis that can highlight relevant and under-recognized genes and gene pathways important in health and disease. Large population based genomic studies have yielded little mechanistic or clinically translatable information. The use of a transcriptomic approach in very carefully defined immune cell populations may circumvent usual genomic difficulties[6] by limiting analysis to transcribed gene product as opposed to the whole genome. Microarray analysis of T cell gene expression profiles identified a signature gene transcription profile that highlights T cell specific genes and transcription factor binding sites that are common in young and successfully aged people[7]. Studies of mRNA signatures associated with successful or failed vaccine responses are less common. It is unknown whether there is a unique T cell or B cell mRNA transcriptomic profile in HIV^+ individuals who respond differentially (very well versus very poorly) to influenza vaccine.

METHODS: Plasma and peripheral blood mononuclear cells will be isolated from HIV positive individuals receiving standard of care seasonal influenza vaccine. Plasma samples from up to one month before vaccination and 3 months post vaccination will be assessed for influenza antibodies by microbead array analysis. Only those with baseline influenza titers less than 2 fold the maximum measurable level with the assay will be considered in order to obtain accurate post-vaccine titer quantification. Individuals with total influenza specific antibody titres 2 fold above background will be categorized as high responders and those with less than a 2 fold increase as low responders. To investigate changes in T and B cell populations associated with influenza vaccine response, B cell and $CD4^+$ T cell populations will be isolated from pre-vaccination peripheral blood mononuclear cells from 10 high responders and 10 low responders via magnetic bead separation. Flow cytometry will be used to confirm population purity, and only bead-selected populations that are >90% pure will be used for array analysis. Only those with HIV viral load less than 50 copies/mL will be considered to avoid the confounding effect of active viral replication on $CD4^+$ T cell function. Likewise, only those with $CD4^+$ T cell counts greater than 200 cells/mL are included to ensure there are enough $CD4^+$ T cells for adequate RNA yield in each sample. Total RNA will be isolated from 10^6 purified cells, DNase treated and hybridized on an Affymetrix® microarray for analysis. Microarray chip reads will be done at The Centre for Applied Genomics, The Hospital for Sick Children.

Gene expression data will be normalized using the Robust Multi-array Average approach to remove outliers, and probe sets will be assessed for discrepant results. The Affymetrix® Transcriptome Analysis Console (TAC) 2.0 Software will be used to assess and annotate gene clusters.

SPECIFIC ROLE FOR SUMMER STUDENT: All patient samples have been collected and response to influenza vaccine will be determined before the start of the summer studentship. In the chosen 20 samples, Ms. Miller will isolate $CD4^+$ T cells and B cells, confirm population purity, and then isolate RNA for cDNA conversion. These are tasks she will perform independently under the supervision of our lab technician. Data is expected back from the Centre for Applied Genomics toward the end of the 12 weeks, and Ms. Miller will be assisting in the data analysis and bioinformatic approaches to the data. She is expected to be familiar with quality control of this type of data, and basic approaches to analysis while working under the tutelage of a senior scientist with bioinformatics experience in our lab.

EXPECTED RESULTS AND IMPLICATIONS: Based on previous results from similar studies that use a more directed analysis, it is likely that high responders will have decreased B cell survival signal pathways and tyrosine kinase genes such as lck[8]. T cell gene signatures of high responders will likely have lower gene expression of exhaustion markers like PD-1 and CD57[9]. While in this small study, gene pathways enriched in one of the groups may not yield statistical significance, this study still provides the opportunity to learn more of the basic biology of T and B cells with this type of analysis and therefore the data are valuable. Better understanding of poor vaccine responses in chronic HIV infection will aid us in the design of improved vaccine formulations or immune modulation that maximizes protection from vaccine preventable diseases. This study has implications not only for individuals living with chronic viral infection but the aging population as a whole.

REFERENCES

1. Armstrong GL, Conn LA, Pinner RW. Trends in infectious disease mortality in the United States during the 20th century. *JAMA* 1999;281(1):61-66.
2. Sasaki S, Sullivan M, Narvaez CF, et al. Limited efficacy of inactivated influenza vaccine in elderly individuals is associated with decreased production of vaccine-specific antibodies. *J Clin Invest.* 2011;121(8):3109-3119.
3. Desai S, Landay A. Early immune senescence in HIV disease. *Current HIV/AIDS Reports.* 2010;7(1):4-10.
4. Malaspina A, Moir S, Orsega SM, et al. Compromised B cell responses to influenza vaccination in HIV-infected individuals. *J Infect Dis.* 2005;191(9):1442-1450.
5. Klein MB, Lu Y, DelBalso L, Cote S, Boivin G. Influenzavirus infection is a primary cause of febrile respiratory illness in HIV-infected adults, despite vaccination. *Clin Infect Dis* 2007;45(2):234-240.
6. Deelen J, Beekman M, Capri M, Franceschi C, Slagboom PE. Identifying the genomic determinants of aging and longevity in human population studies: progress and challenges. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology.* 2013;35(4):386-396.
7. Remondini D, Salvioli S, Francesconi M, et al. Complex patterns of gene expression in human T cells during in vivo aging. *Molecular bioSystems.* 2010;6(10):1983-1992.

8. Kardava L, Moir S, Shah N, et al. Abnormal B cell memory subsets dominate HIV-specific responses in infected individuals. *J Clin Invest* 2014;124(7):3252-3262.
9. Wherry EJ, Ha SJ, Kaech SM, et al. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity.* 2007;27(4):670-684.