Research Rotations

After you have completed a rotation it is expected that you will (1) give an oral lab presentation of your project; and (2) write up a short report of your rotation. (A sample report is below.) The report should be submitted with the evaluation form (below) to the rotation mentor at or near the end of the rotation. The mentor will review the report and complete form.

You should have an "exit" interview with the mentor to discuss the rotation, going over the evaluation and report. The interview is meant to be constructive and to give useful feedback to you. It is expected that the research advisor will be honest and indicate the degree to which he/she is interested in having you as a student in his/her lab. You may also want to indicate your degree of interest in joining the lab. After both you and the mentor have signed the form, email it along with the report to mstp@case.edu.

Evaluation forms signed by the student and mentor should be submitted for at least three rotations. Typically the rotation report and evaluation should be completed and returned to the MSTP office within two weeks of the end of the rotation. Timely submission of these materials is required to get a grade of "Pass" in MSTP 400.

ROTATION EVALUATION: MEDICAL SCIENTIST TRAINING PROGRAM

STUDENT NAME _____

FACULTY NAME _____ DEPT._____

DATES OF ROTATION _____

1. Did the student spend the expected time per week in the lab? (Summer: Full time for 4-6 weeks; Fall or Spring: ~20 hrs/week for 8-12 weeks) Yes_____   No_____

2. Did the student learn any new techniques?   Yes_____   No_____

3. Did the student get any new results or data?   Yes_____   No_____

4. Did the student do a lab group meeting presentation or other presentation of their rotation project?   Yes_____   No_____

5. How would you rate the students overall performance in this rotation?

    Poor_____   Average _____   Good _____   Excellent _____

6. Based on this student's performance and compatibility with the laboratory as a whole would this student be suitable for placement in your laboratory? Yes_____ No_____

7. Comments. Please address the following questions (**add additional pages if necessary**).

    What are the strengths of the student? What areas need improvement? Other comments or information?

_____   _____
           Faculty Signature   Student Signature

_____   _____
                 Date   MSTP Director

After the student and faculty member have reviewed and signed this form, it should be emailed, along with the report, to mstp@case.edu. Students should submit a rotation evaluation within two weeks of the end of the rotation.

SAMPLE ROTATION REPORT

Effect of a broad-spectrum vaccine on evolutionary rate of simian-human immunodeficiency viral protein gp120 in a macaque pathogenesis model

MSTP student: _____; Preceptors: Dr. Gabrielle Nickel and Dr. Eric J. Arts

Abstract/Summary

From July – August 2013, I worked under the direction of Dr. Gabrielle Nickel, a post-doctorate researcher in the laboratory of Dr. Eric J. Arts, to analyze sequencing data from a collaborator's project investigating population-level changes in the viral sequence encoding glycoprotein 120 (gp120) of simian human immunodeficiency virus (SHIV) in a macaque pathogenesis model. To assess the effect of a broad-spectrum vaccine on the evolution and infectivity of the virus, the collaborator vaccinated monkeys with replication-defective SHIV pseudovirus representing the env variants of 10 different HIV-1 subtypes. As controls, animals were vaccinated with pseudovirus expressing only the B-subtype env variant. These monkeys were then infected with a SHIV clone expressing the B-subtype env variant. After 1 and 3 weeks, blood lymphocytes were collected from each monkey, and the gp120 region in a sample provirus population was PCR-amplified and deep-sequenced. As a measure of evolution in gp120 over time, we tracked changes in the frequency of predicted N-glycosylation sites. Preliminary results show differences in the rate of loss or gain of N-glycosylation sites in gp120 between different animals, presumably due to differential selection pressure of the vaccination treatment on the virus, though at this point we are still blinded to the vaccination treatment of each animal.

Introduction

The development of an effective preventive HIV-1 vaccine has proven to be an elusive goal for many reasons, including the heterogeneity of the virus, the high mutation rate of the viral genome, and the inaccessibility of virion surface proteins to host antibodies. However, promising results from a landmark 2009 HIV-vaccine trial (RV144) that lowered the risk of acquiring HIV-1 by 31% have spurred renewed attention on this goal, with specific focus on gp120.

Gp120, a virion surface envelope glycoprotein encoded by the env gene, plays an essential role in HIV-1 infectivity, by binding the CD4 receptor and co-receptor of target CD4 T-lymphocytes and mediating viral entry. Glycosylation of this protein at arginine residues is important for this function, as the N-linked glycans are essential for the receptor binding-induced conformational changes in gp120 necessary for membrane fusion. In addition, glycosylation provides a "glycan shield" that masks epitopes from the host immune system. This immune escape strategy makes the sequence encoding gp120 one of the most variable regions in the HIV-1 genome.

To measure the efficacy of a broad spectrum vaccine, our collaborator vaccinated 6 monkeys with a combination of pseudovirus representing the gp120 variants of each of 10 HIV-1 subtypes. After infection with the B-subtype SHIV, the gp120 region of a sample provirus population was sequenced at various time-points post-infection. I contributed to the sequence analysis for 2 of the 6 samples. As the reads covered specific sub-parts of the full-length gp120 sequence, I first mapped each read to the known, full-length sequence using an alignment tool, MUSCLE. Next, I calculated the frequency of predicted N-linked glycosylation sites from the translated sequence reads, by developing and running a customized Perl script which finds in the sequence all matches to the pattern predictive for N-linked glycoslyation sites (Nx[ST]).

Results

Animal 1				Animal 2			
Week 1		Week 3		Week 1		Week 3	
Position	Frequency	Position	Frequency	Site	Frequency	Site	Frequency
…	…	…	…	…	…	…	…

Table 1. [unpublished data redacted] Positions of predicted N-linked glycosylation sites and their frequencies in a sample population of gp120 sequences, 1 week and 3 weeks after infection, for 2 different animals. In red and green are positions lost and gained, respectively, between the two time-points.

The distribution of predicted N-linked glycosylation sites in gp120 is shown for two different animals in Table 1. In Animal 1, between weeks 1 and 3, X sites were lost, and X sites were gained in the population of gp120 sequences. In contrast, in Animal 2 in the same period of time, X sites were lost in the population of gp120 sequences, and X were gained. Though quite preliminary, these results may be consistent with our hypothesis that the rate of evolution in gp120 is accelerated in subjects of the broad-spectrum vaccine, because of higher selection pressure on the virus from antibodies against all gp120 subtype variants compared to antibodies against a single gp120 subtype variant.

Future directions

I will confirm each monkey's vaccination treatment to associate rate of change in the distribution of N-linked glycosylation sites with vaccination treatment. I am currently working with Dr. Nickel to assess the genetic diversity of the gp120 populations at each time-point using the Kimura 2-paramter model, which estimates the evolutionary distance between two sequences from the frequency of nucleotide substitutions. We expect that genetic diversity of gp120 populations will follow similar trends as the rate of change in the distribution of N-linked glycosylation sites, comparing the subjects of the broad-spectrum vaccine with controls. In addition, we will

correlate evolutionary rate in the gp120 sequence with viral load measurements to assess whether accelerated evolutionary rate indeed contributed to increased viral fitness in this study.