Assessment of T cell chemotaxis to S1P

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Introduction: Migration of leukocytes in response to chemical gradients, chemotaxis, is dependent on many factors, including cell type, surface markers, the chemoattractant, etc. Sphinogsine-1-phosphate (S1P) is a chemoattractant playing a large role in migrating activated T cells out of lymph nodes by binding to their S1P receptor, S1P1. The importance of the egress in T cells from lymph nodes is highlighted by pharmacological disruption of this migration can lead to immune dampening and thus control of multiple sclerosis, an autoimmune disease. In the case of human immunodeficiency virus (HIV), it has been shown that HIV downregulates S1P1 surface expression, effectively inhibiting chemotaxis. Our experiments attempt to study a particular HIV-encoded protein, Nef in S1P-elicited T cell migration, and to optimize the conditions for assessing T cell migration in response to S1P. **Methods:** In our Transwell migration assays, migration of serum-starved SupT1 cells was induced using various concentrations of S1P bound to delipidated bovine serum albumin (BSA). Before migration, cells were labeled using Calcein AM. Cells were allowed to migrate for 2-4 hours at 37°C in serum-free media. After migration, fluorescence intensity was measured using a CLARIOstar microplate reader. **Results:** S1P showed a direct dose-dependent response to SupT1 cell migration from 0 to 100 nM S1P. Optimization of the migration showed that both number of trans-migrated cells and those still present within the transwell filter were significant indicators of SupT1 migration. **Conclusion:** S1P's chemoattractant ability is prevalent in the migration of SupT1 cells in concentrations lower than 125nM. Because we have inducible systems for HIV-Nef expression established in this cell line, these data are useful for testing the role of Nef in HIV-mediated T cell retention.