Comparing Virus Use of Host Receptors by Shotgun Mutagenesis

Viral use of Host Cell Receptors

The chemokine receptor CCR5 is the principal coreceptor for HIV. By interacting with viral Envelope, CCR5 facilitates virus binding and entry to the host cell. Blocking coreceptor function protects against HIV infection without apparent deleterious health effects. This makes CCR5 a particularly attractive target for the control of HIV infection, and several new drugs that inhibit viral fusion via CCR5 are now entering the market. However, strains of HIV that are resistant to first-generation CCR5 inhibitors have already been identified. Remarkably, these strains appear to have developed resistance because they have changed the way in which they interact with CCR5, and now utilize receptor structures that are not inhibited by the drug. Investigating the structural basis of viral-receptor interactions is therefore important for understanding the mechanism of viral drug resistance, and for designing new drugs that are less susceptible to viral evasion. However, membrane proteins such as CCR5 are unsuited to direct structural analysis by crystallography or NMR, making their molecular interactions particularly difficult to characterize.

Shotgun Mutagenesis

Shotgun Mutagenesis is a method for high-resolution mapping of protein interactions, such as between viral proteins and their receptors. It involves comprehensively analyzing the effects of mutation at every amino acid position in a target protein. While site-directed mutagenesis is the most common method for identifying local binding regions of membrane proteins, conventional strategies are slow and laborious, usually requiring months to years to map even small portions of a protein. Shotgun Mutagenesis uses a unique high-throughput expression strategy that enables hundreds to thousands of individual variants of a target protein, each bearing a unique mutation, to be expressed simultaneously for rapid functional analysis. Importantly, mutated proteins can be evaluated using standard functional assays, such as HIV infection of living cells. Shotgun Mutagenesis thus provides large sets of functional experimental data to support the construction of high-quality virus-receptor interaction models.

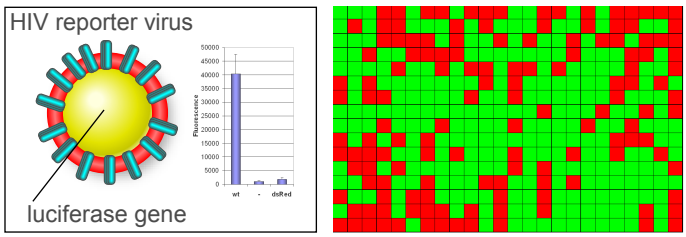

Figure 1. Infection of cells expressing CCR5 point mutants. HIV-1 and SIV strains carrying a luciferase reporter gene were used to infect a library of approximately 1,500 CCR5 mutants. Failure of viral strains to infect a particular well indicate an amino acid that is likely to be critical to the virus-receptor interaction. Results from control wells are graphed, and results for one microplate of infection results are color-coded by comparison to infection using wild type CCR5.

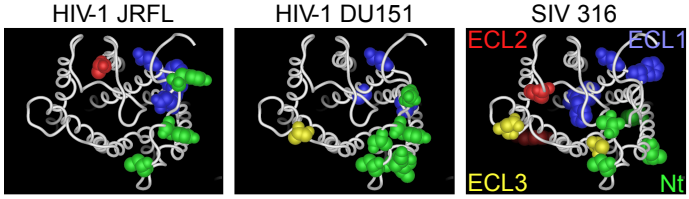

Figure 2. Comparison of amino acids used by two strains of HIV-1 and a strain of SIV. The extracellular surface (top-down view) of CCR5 is shown. While many of the viral binding regions of CCR5 are common to all three viruses (N-terminus and ECL1), significant molecular differences are apparent (particularly in ECL2 and ECL3). Nt: N-terminus, ECL: Extracellular Loop.

Technical Description

Shotgun Mutagenesis was used to compare the structures of CCR5 that support viral entry by two different HIV-1 strains (JRFL and DU151) and a strain of the simian immunodeficiency virus (SIV 316) that is commonly used to study HIV infection. A library of approximately 1,500 CCR5 clones in which every amino acid was mutated at least once was expressed in living eukaryotic cells co-expressing the HIV receptor CD4. Full-length translation of each mutant and its expression on the cell surface was verified by immunofluorescent staining. Cells were then infected with one of the three viruses, each engineered to deliver a luciferase reporter gene upon cell entry. Infection was allowed to proceed for two days, after which cells were lysed and assayed for luciferase expression (Figure 1). CCR5 amino acids that are utilized for entry by each virus were identified when a point mutation eliminated cell entry despite continued cell surface expression of the receptor. Critical amino acids were mapped onto a three-dimensional model of CCR5, highlighting potentially important differences in the way in which the three viruses interact with CCR5 (Figure 2). These differences suggest that CCR5 inhibitors that protect against one strain (e.g. clade B strains such as JRFL, which are prevalent in the U.S.) might not necessarily provide protection against other strains (e.g. clade C strains such as DU151, which are prevalent in Africa and Asia). Conversely, the identification of conserved binding structures that appear to be utilized by all three viruses (e.g. on the N-terminus or ECL1) may be useful for designing new inhibitors that block common structures of CCR5 that are critical to its function as a viral coreceptor.

Contact Us

Shotgun Mutagenesis mapping, including comprehensive mutation of user-specified genes, data collection, and structural analyses, are provided to customers on a fee-for-service basis. For more information contact us at:

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