

Abstract

Since its discovery, 30 years ago, the Human Immunodeficiency Virus (HIV), the etiological cause of AIDS, has killed more than 25 million people and a further 40 million are living with the disease. The HIV-1 Envelope protein (Env) facilitates HIV-1 entry into susceptible target cells by fusion at the cell membrane upon binding to the CD4 receptor and to one of two coreceptors, CCR5 or CXCR4. In vivo main target cells for HIV are CD4⁺ T cells, macrophages and dendritic cells (DCs). In this doctoral research, we focused on entry and transmission of primary HIV strains in primary target cells for HIV in vivo. In the first part of this work we present an in-house recombinant viral assay (RVA) to assess viral coreceptor usage and its validation using a wide panel of primary strains of various subtypes. With the advent of entry inhibitors and particularly Maraviroc, a CCR5-binding molecule, assessing viral co-receptor usage has become a necessity to predict drug efficacy and to avoid the emergence of CXCR4-using strains which are more cytopathic and associated with disease progression. Co-receptor usage may be determined genotypically from the sequence of the Env V3-loop or phenotypically by infecting indicator cell lines expressing CD4 and one of the two coreceptors, CCR5 or CXCR4 with recombinant or pseudotyped viruses containing portions of the Env accounting for tropism. We compared tropism measured phenotypically using our in-house RVA to the most widely used genotypic prediction tools, Geno2Pheno[coreceptor] and webPSSM. In this study we included all the major HIV-1 subtypes and to our knowledge it has the widest range of HIV-1 subtypes and circulating recombinant forms (CRFs) in a single study. Discordances were observed for non-B subtypes. Experiments using CCR5 or CXCR4 antagonists confirmed the specificity of the RVA and indicated that genotypic prediction tools overestimated CXCR4 usage for CRF01_AE and CRF02_AG. Applying other rules described by others for these CRFs improved concordance, indicating that further subtype-specific tuning would improve their performance. The second part of this work addresses the impact of polymorphisms in the cytoplasmic tail of Env (gp41 CT) on infection of CD4⁺ T cells and primary monocyte-derived-macrophages (MDMs). The gp41-CT is involved in Env incorporation into virions and contains intracellular trafficking motifs that mediate its extensive cycling through the endocytic compartment. Gp41-CT truncations impair propagative infection in some cell types (non-permissive cells) including primary CD4⁺ T cells and macrophages. We used a comparative approach based on NL4-3/Env and NL4-3/Env ectodomain recombinant virus pairs: in this system, either full patient-derived Envs or isogenic patient-derived Env ectodomains are cloned in an otherwise constant NL4-3- derived backbone by homologous recombination. Subtype B and C Envs were compared. Subtype C gp41-CT impaired viral replication compared to the NL4-3 gp41-CT in CD4⁺ T cells (10/13 cases) but not in MDMs. For other subtypes, isogenic viruses replicated similarly in both cell types. Addition of extracellular deoxynucleosides to MDMs did not impact viral replication. Sequence analysis revealed that most domains involved in Env intracellular trafficking were conserved. Whereas subtype B gp41-CTs were less polymorphic, subtype C was comparatively highly polymorphic and dileucine (LL) motifs were often disrupted. Restoring the C-terminal LL motif restored viral replication in CD4⁺ T cells,

suggesting it plays a role on the level of Env incorporated, as this motif is involved in Env internalization and cycling