Understanding mechanisms of HIV-1 entry into cells

Paul R Gorry

Centre for Biomedical Research, Burnet Institute, GPO Box 2284, Melbourne, Vic. 3001, Australia
Department of Infectious Diseases, Monash University Central Clinical School, Melbourne, Vic., Australia
Department of Microbiology and Immunology, University of Melbourne, Parkville, Vic., Australia
Tel: +61 3 9282 2129
Fax: +61 3 9282 2100
Email: gorry@burnet.edu.au

Human immunodeficiency virus type 1 (HIV-1) attaches to cells by the stepwise interaction of its envelope glycoproteins (Env), which exist as trimers that stud the exterior of the virus particle, with cellular CD4 and a coreceptor, principally either of the chemokine receptors CCR5 or CXCR4. Virus entry into cells then proceeds via exposure of a viral fusion peptide, and fusion between the viral and cellular membranes. Adaptability in Env conformation is a hallmark of HIV-1, which permits the virus to escape the humoral immune response. HIV-1 may also harness this power of adaptability to alter its target cell tropism and develop resistance to CCR5 antagonist HIV-1 entry inhibitors. Our work has shown that this may occur through a more efficient interaction between the Env glycoproteins and cellular receptors, as well as by an altered (but not necessarily more efficient) mechanism of interaction. Understanding the complexity of these interactions is pivotal for elucidating the molecular determinants of HIV-1 pathogenesis.

HIV-1 tropism refers to the ability of virus to establish infection in alternative immune cell types (reviewed by Gorry and Ancuta[1]). HIV-1 isolates are frequently classified as being macrophage (M)-tropic, T-cell tropic, or dual-tropic. M-tropic viruses can infect primary CD4+ T-cells and macrophages and principally use CCR5 as coreceptor; T-cell tropic viruses can infect CD4+ T-cells and T-cell lines via CXCR4; and dual-tropic viruses can infect the three different cell types and can use CCR5 and/or CXCR4[1]. Changes in viral tropism for CD4+ cells may exert an influence on HIV-1 pathogenesis in a number of ways. For example, enhancement of M-tropism is important for pathogenicity in subjects exclusively maintaining CCR5-using (R5) viruses to late stages of infection[2], neurotropic variants may emerge and cause neurological impairment[3], and CXCR4-using (X4) variants, which can arise at late stages of infection in 40–50% of patients[4,5], have expanded tropism for CD4+ T-cell subsets[6].

Coreceptor usage, as defined by the ability of HIV-1 isolates to interact with CCR5 and/or CXCR4 in transfected cell lines is frequently used to define HIV-1 tropism. For example, R5 viruses are often thought of as intrinsically M-tropic (reviewed by Gorry and Ancuta[1] and Gorry *et al.*[7]). However, although most M-tropic viruses use CCR5 for HIV-1 entry, not all R5 viruses are M-tropic. In fact, most R5 HIV-1 strains cannot enter macrophages[8,9]. Our work has shown that M-tropic HIV-1 viruses are more likely to be isolated from the central nervous system (CNS), where the immune privileged nature of the CNS permits adaptive alterations in Env to enable the virus to scavenge low levels of CD4 that are expressed on macrophages, or which enable an altered mechanism of CCR5 binding[10–13]. Nonetheless, M-tropic HIV-1 variants do emerge in blood and can be detected in peripheral tissues, although the frequency is much lower as compared to CNS compartments such as the brain and cerebrospinal fluid (CSF)[2]. In contrast to CNS-derived M-tropic viruses, however, we have shown that those located in the periphery tend to have a more efficient interaction with CCR5, which may or may not occur in tandem with a more efficient interaction with CD4[14,15]. The determinants underlying M-tropism of R5 HIV-1 strains is therefore complex, and may be influenced by different selection pressures exerted by compartmentalisation in different anatomical sites. In addition, we have shown that a subset of highly M-tropic viruses can enter macrophages via CXCR4[12,14]. Here, M-tropism is dictated by a more efficient and altered mechanism of interaction between Env and CXCR4 rather than more efficient CD4 binding. This collective body of work illustrates that the determinants of HIV-1 tropism are more complex than the coreceptor specificity of the virus.

CCR5 antagonists are allosteric inhibitors of HIV-1 entry. Maraviroc (MVC) is the only CCR5 antagonist licensed for clinical use, although other experimental CCR5 antagonists include vicriviroc (VVC) and aplaviroc (APL)[16]. As opposed to a competitive mechanism of inhibition, CCR5 antagonists prevent Env binding to CCR5 by altering the conformation of CCR5 such that it is no longer recognised by HIV-1.

The relationship between efficiency of the Env-CCR5 interaction and M-tropism can also be inferred from our recent studies of HIV-1 resistance to MVC[17]. Here, studies of a MVC-resistant strain of HIV-1 that was generated *in vitro* showed that utilisation of MVC-occupied CCR5 by this virus was less efficient than that of unoccupied CCR5, but that the ability to interact with CD4 was unaffected by MVC. Whilst the MVC-resistant variant could efficiently enter CD4+ T-cells in the presence of MVC, its M-tropic properties were abolished due to a less efficient interaction with CCR5. We subsequently confirmed these *in vitro* findings using primary MVC-resistant viruses isolated

from plasma of subjects who developed resistance during phase III clinical trials of MVC[18,19]. Consistent with our observations, other studies of HIV-1 resistance to APL demonstrated tropism alterations for CD4+ T-cell subsets by an APL-resistant HIV-1 strain due to a less efficient interaction with CCR5, characterised by a tropism shift towards effector memory T-cells and relative sparing of central memory T-cells[20].

Together, these studies indicate that a reduction in the efficiency of the interaction between Env and CCR5 can attenuate HIV-1 entry of blood-derived HIV-1 strains into primary CD4+ cells that have limiting CCR5 levels, including macrophages. Whilst an enhanced interaction between Env and CD4 appears to be the major pathway to efficient macrophage entry by brain-derived viruses, M-tropic viruses isolated from blood appear to have distinctive Env conformations that alter the mechanism of CCR5 binding, in addition to augmenting CD4 binding, to efficiently enter macrophages. These concepts that are touched upon in the preceding sections are explored in greater detail in two recent review articles from my laboratory[1,7]. Due to their longevity in tissues, HIV-1 infected macrophages are presently a major obstacle for efforts aiming to eradicate HIV-1 from the body. Understanding the complex interactions between Env and cellular receptors that underlie M-tropism of HIV-1 strains will shed light on understanding viral persistence in the macrophage HIV-1 reservoir.

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Biography

Professor Paul Gorry heads the HIV Molecular Pathogenesis Laboratory at the Burnet Institute in Melbourne, and is Deputy Head of the Institute's Centre for Biomedical Research. He also holds the position of Burnet Senior Principal Research Fellow, and the appointment of the Institute's Principal for Immunity, Vaccines and Immunisation. He is an Adjunct Professor in Medicine (Infectious Diseases) at Monash University, and an Australian Research Council Future Fellow. Professor Gorry obtained his PhD in 1998 and then undertook postdoctoral training at the Dana-Farber Cancer Institute, Harvard Medical School, between 1999 and 2002, before returning to Australia to establish his laboratory at the Burnet Institute. His research has been funded over the past 12 years by project grants and fellowships from the Australian National Health and Medical Research Council, the US National Institutes of Health, the Australian Research Council, and the Australian Centre for HIV and Hepatitis Virology Research. A molecular virologist, his research broadly aims to understand mechanisms of HIV-1 entry into cells. Specifically his research investigates: (i) the entry mechanisms governing cellular tropism of HIV-1; (ii) mechanisms involved in HIV-1 resistance to CCR5 antagonists; (iii) mechanisms contributing to HIV-1 pathogenesis; and (iv) development of new HIV-1 diagnostics and entry inhibitors.