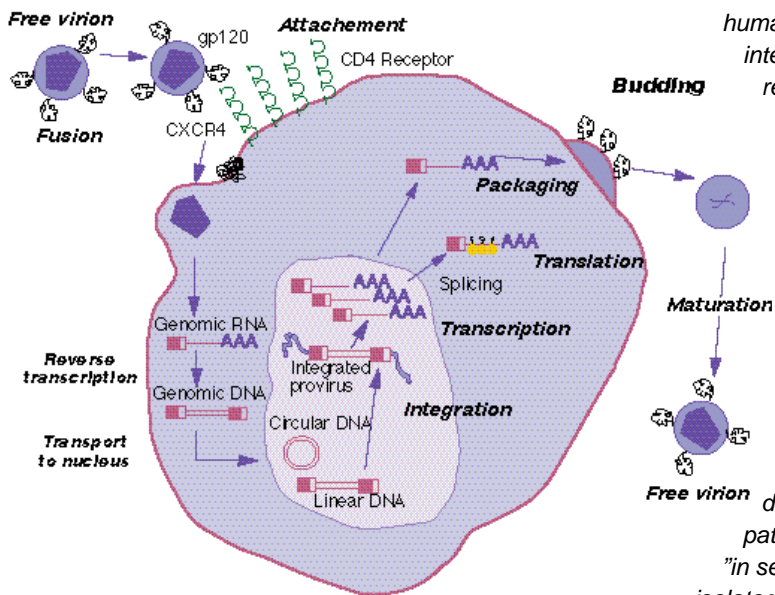


RETROVIROLOGY

Introduction



No group of viruses has received as much attention from scientists in recent years as the retroviruses. All humans carry human endogenous retrovirus (HERV) sequences as an integral part of their genomes (1). In contrast, exogenous retroviruses occur only in those cells of an infected individual which support virus entry and replication.

Several exogenous retroviruses are etiologic agents of severe diseases in man. Research studies have revealed that the human T cell leukemia virus (HTLV), first identified in 1980, is associated with a clinical aggressive form of adult T cell leukemia (ATL) and with the degenerative neuromuscular diseases, tropical spastic paraparesis and HTLV-I-associated myelopathy (TSP/HAM) (2). The human immunodeficiency virus (HIV), first characterized in 1983, is the causative agent of acquired immunodeficiency syndrome (AIDS) and associated diseases (3). Of the two forms, HIV-2 appears to be less pathogenic than HIV-1 in man. Other retroviruses are still "in search of a disease". The human foamy virus (HFV), first isolated in 1971, is apparently benign in humans. However, fatal encephalopathy and myopathy have been described in transgenic mice expressing the entire HFV or only its regulatory genes (4). All these retroviruses share many biological and molecular characteristics. These include the presence of viral-encoded regulatory proteins that function at both transcriptional and post-transcriptional levels and which are found only in complex retroviruses.

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There is evidence of specific requirements favoring the establishment of infection. A major force in promoting the adaptation of a virus to its host is the presence of a high affinity receptor. Therefore, identification of cell surface molecules that act as portals of virus entry into human cells is of major research importance. This will enable understanding of the mechanism of virus tropism and infection, the development of drugs aimed at preventing infection of target cells, and yield information useful for the development of vectors for gene therapy.

The Cell Surface Receptors

Retroviruses utilize a variety of apparently unrelated cell surface receptors to initiate infection (5). For example, transporter molecules with multiple membrane-spanning domains act as receptors for several non-human retroviruses. At the end of the last decade, one of the best defined receptors was that for HIV. The primary high-affinity cellular receptor for most HIV isolates on T cells is the CD4 molecule (6). CD4 is a member of the immunoglobulin (Ig) superfamily. It consists of an extracellular region of 370 amino acids organized in four domains (D1 to D4); a hydrophobic membrane-spanning region of 25 amino acids; and a highly charged cytoplasmic tail of 38 amino acids (7). While D2, D3, and D4 domains of CD4 resemble the human immunoglobulin constant regions, D1 shows structural homology with Ig V regions composed of three hypervariable regions, termed complementarity-determining regions (CDRs) (8, 9). Anti-CD4 monoclonal antibodies (such as clone 13B8-2) have been useful for investigating the role of CD4 regions in HIV replication (10–13).



Expression of CD4 is necessary but not sufficient for cell-free infection of human cells with HIV. Binding to CD4 is followed by a cascade of post-binding events. These include recruitment of the newly discovered seven-span transmembrane (7TM) G protein-coupled chemokine co-receptors (CXCR4, CCR5) which forms the fusion complex at the cell membrane (14, 15). In contrast to HIV, *in vitro* cell-free viral infection by HTLV is extremely difficult to achieve although a low level of successful infection could be obtained for both primary cells and cell lines. HTLV is transmitted almost exclusively via cell-to-cell contact. On the basis of receptor interference assays, HTLV-1, HTLV-2, chimpanzee T cell leukemia virus, and simian T cell leukemia virus have been shown to have a receptor in common. Although the cellular receptor for HTLV has a wide species and cell type distribution, as determined by *in vitro* studies, it has yet to be fully characterized. Similarly, the receptors for HFV remain to be identified.

Different Strategies to Replicate

Other than selection of receptors, the biological forces that promote the adaptation of retroviruses to their hosts are poorly understood. It is worth noting that although HIV can enter resting T cells, cell activation is required to ensure complete reverse transcription and/or integration and to induce subsequent progeny virus production. Integration provides an essential step in the retroviral life cycle by inserting a DNA copy of the viral genome (called a provirus) into the host genomic DNA. In contrast to HIV, cell division is required for HTLV provirus integration. Provirus transcription is under control of recognition sequences that bind host cell transcription factors, and which are located in the 5' long-terminal repeat (LTR).

Different strategies have been developed by retroviruses to stimulate their transcription. Tat (the HIV trans-activator viral protein) binds to the TAR element which forms a stable stem-loop hairpin at the 5'-end of viral mRNAs. Through this region, Tat appears to influence both transcription and elongation. Tax (the HTLV trans-activator protein) activates cellular transcription factors such as CREB, CREM, NF- κ B and SRF through indirect binding to each specific DNA sequence. It can also activate nuclear translocation of NF- κ B by interacting with the NF- κ B-negative regulator I κ B. Finally, Bel1 (the HFV trans-activator protein) is a DNA-binding protein.

Detection Systems

The readout systems commonly used to study virus transcription use a variety of methods:

- ◆ Reverse transcriptase-polymerase chain reaction to monitor mRNA expression.
- ◆ Promoter activation assays in transfected cells using a reporter gene expression vector.
- ◆ Quantification of progeny virus production.

Virus production in cell culture supernatants can also be monitored several ways:

- ◆ Measurement of reverse transcriptase (RT) activity using a synthetic template primer and 3H-thymidine under experimental conditions which permit the RT to neo-synthesize radioactive DNA.
- ◆ A convenient non-radioactive method consists of measuring the concentration of virus core antigen in cell culture supernatants. The availability of a sensitive antigen capture assay is of great interest for the detection of HTLV in infected cell cultures since these cells usually have very low cell-free virus production (16).

Molecular Crosstalk between Viruses and Host Cells

In the quest for a better understanding of the physiopathology of retrovirus infections, it has become apparent that retrovirus replication depends on a plethora of highly specific interactions with the host cell resulting in the subversion of multiple cellular signal transduction pathways. Understanding the molecular cross-talk that regulates the relationship between the host cell and virus is currently the objective of many researchers. A surprisingly obvious concept has emerged recently, based on the idea that bound viruses may act as ligands able to stimulate receptor function. As a direct consequence, viruses may be expected to modulate the homeostasis of the target cells following receptor binding. For example, research studies have shown that HIV exhibits a tremendous capacity to modulate kinase activation and nuclear translocation of transcription factors, such as AP-1 and NF- κ B, following binding to CD4 (17-19). Similarly, viral envelope interaction with the co-receptors was found recently to activate kinases (20). Such events might operate in two opposing ways. First, they might contribute to cell activation and proliferation required for virus production. Second, they might favor anergy or apoptosis. In both cases, research studies have shown that these events can cause dramatic changes in the progression of disease in infected patients (21). Defining this molecular cross-talk in depth is needed to gain insight into several puzzling features of retrovirus pathogenesis.



Researching Retrovirus-induced Diseases by Cell Surface Marker Monitoring

One approach to understanding how viruses act on the immune system is the study of quantitative variations in distinct cell subsets and associated changes in expression of cell-surface markers. In some cases this approach can be considered as a basic research tool. For example, preferential expansion of the V γ 7 T cell receptor (TCR) γ T cell subset in patients suffering from acute-onset type I diabetes has been recently reported in research studies. This expansion correlates with expression of a human endogenous retrovirus envelope protein (encoded by HERV-K) which acts as a superantigen. Thus, this superantigen constitutes a candidate for the induction of an autoimmune T cell response causing type I diabetes (22). The V γ 7 TCR expression may therefore be further studied for its ability to represent a marker of type I diabetes.

Another example can be found in the HTLV-I model. A common pathological finding in HTLV-I-associated diseases is the infiltration of HTLV-I infected T cells into various organs. This process is favored by adhesion molecule-mediated interactions between circulating T cells and endothelial cells. Another research study reported that HTLV-I transformed T cells express high levels of VCAM-1 (CD106), a phenotypic change which is linked to viral Tax protein transactivation of VCAM-1 (23).

In other situations, the phenotypic characterization of cell subsets is used to explore a patients' immune system. For example, depletion of CD4 γ T cells is the hallmark of the immune system dysfunction characteristic of AIDS. Even in early stages of infection, lymphocytes of infected patients show functional immunological alteration despite signs of chronic immune activation. Consequently, three-color flow cytometry performed with markers such as CD4, CD8, CD25, CD38, CD45RO, CD45RA, CD69, or HLA-DR has been widely used in research to explore the decline in CD4 γ T cell counts and T cell immunity of HIV-infected patients.

Recently, potent cocktails of antiviral drugs have led to the reduction of HIV to almost undetectable levels in the blood of many infected patients and to partial restoration of CD4 γ T cell numbers (24, 25). However, these patients still harbor latent virus in a small number of their T cells (reservoir) which can be induced to replicate when these cells become immunologically active. Moreover, fully rebuilding an HIV-ravaged immune system is not expected to be possible using the treatments available today. Many researchers wish to learn more about the physiopathology of HIV infection. They believe that analysis of cell-surface markers can be used to categorize infected cells as memory or naive subsets and want to analyze the functional abilities of these cells. One can assume that the next generation of drugs aimed at preventing or treating retrovirus-induced diseases will not be designed without taking into account both partners, namely the retroviruses and the immune system.

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