Transformation by deltaretroviruses: mechanisms and therapies

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Abstract

Human T-lymphotropic virus type 1 (HTLV-1) and bovine leukemia virus (BLV) are two closely related deltaretroviruses inducing hematological diseases in human and ruminants. HTLV-1 infects about 25 million subjects worldwide and causes Adult T-cell leukemia-lymphoma (ATLL) or HAM/TSP (HTLV-induced myelopathy - tropical spastic paraparesis). BLV is the etiological agent of enzootic bovine leukemia. HTLV-1 and BLV have developed strategies to subvert hosts’ immune surveillance. Understanding these mechanisms has allowed development of novel therapeutic approaches.

Keywords: retrovirus, oncogene, histone deacetylase, vaccine, checkpoint
INTRODUCTION

The deltaretrovirus genus of the Retroviridae family comprises a series of related viruses infecting lymphocytes of human, primates and ruminants (1, 2). The prototypes of this genus are HTLV-1 (human T-lymphotropic virus type 1) and BLV (bovine leukemia virus). The pathologies induced by these two viruses share a series of common features (Table 1).

The host range of both viruses is quite distinct: HTLV-1 infects human and primates whereas BLV naturally persists in cattle and water buffalo. There is currently no evidence for infection of human with BLV, even in countries with high prevalence or in regions where consumption of raw milk and meat from infected cows is a habit. Both viruses have a worldwide distribution with a higher prevalence in tropical regions for HTLV-1. On the other hand, BLV has been eradicated from EU by selective culling. Generally, infection remains clinically asymptomatic over extended periods (e.g. up to 70 years in human). A minority of infected hosts (about 4-5%) may develop a leukemia/lymphoma called ATL (Adult T-cell Leukemia) and EBL (Enzootic Bovine Leukosis) in human and bovine species respectively. HTLV-1 infected subjects can also be affected by a neuropathological disease called HAM/TSP (HTLV-associated myelopathy / Tropical Spastic paraparesis) characterized by a progressive degeneration of the spinal cord.

Table 1: Prevalence, distribution, pathologies and cell type specificities of HTLV-1 and BLV

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<th>HTLV-1</th>
<th>BLV</th>
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<tbody>
<tr>
<td>Prevalence and distribution</td>
<td>20 million people infected worldwide, mostly in endemic regions in Japan, Caribbean, South America and Africa</td>
<td>- Naturally infects cattle and water buffalo</td>
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<td></td>
<td>- Worldwide distribution but eradicated from Europe</td>
<td>- Worldwidely distribution but eradicated from Europe</td>
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<td>Viral persistence</td>
<td>Asymptomatic in 95 % of infected subjects</td>
<td>Mostly asymptomatic but one third of infected cows develop a benign persistent lymphocytosis</td>
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<td>Pathologies</td>
<td>4 % of Adult T-cell leukemia (ATL) or HTLV-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) after 20-70 years of clinical latency</td>
<td>3-5 % of leukemia or lymphoma after 4-10 years latency period</td>
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<tr>
<td>Cell type specificity</td>
<td>CD4 + CD45RO + (memory) T cell and CD8 + T cell</td>
<td>CD5+ CD11+ B lymphocytes</td>
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HTLV-1 and BLV genomic organization and gene functions are similar. In addition to the structural gag, pol and env genes required for the synthesis of the viral particle, both viral genomes contain a particular region pertaining to the deltaretrovirus genus (Figure 1). Located between the envelope gene and the 3’ long terminal repeat (LTR), these sequences encode a series of regulatory genes. In particular, Tax and Rex are transcriptional and post-transcriptional activators of viral expression (3, 4). BLV R3/G4 and HTLV-1 p12/p13/p30 are accessory genes that are dispensable for viral infectivity but required for efficient replication (5, 6). A marked difference between both viruses is expression by HTLV-1 of HBZ, a 3’ LTR-directed antisense-transcribed factor (7) important for oncogenicity (8). Despite intensive efforts, no such antisense RNA has ever been cloned in BLV. Instead, the BLV genome has recently been shown to express viral microRNAs from internal pol III promoters (9).

Similarity between both viral systems has allowed further understanding of the mechanisms of pathogenesis in a perspective of comparative virology. For example, understanding of the involvement of viral genetic determinants in infection, persistence and pathogenesis was pioneering in the field of BLV (10). In contrast, the fundamental mechanisms of viral expression, metabolic pathways or immune response regulation have been far better characterized in the HTLV-1 system (11). Similarity between the two viruses in terms of pathogenic mechanisms has also been instrumental in developing novel therapeutic approaches. Here, we develop two novel strategies issued from understanding of viral mechanisms of pathogenesis.

Figure 1. Genomic structure of the BLV and HTLV-1 genomes. LTR (long terminal repeat), GAG (group specific antigen), ENV (envelope), TAX (transcriptional activator protein of the X region) and REX (post-transcriptional regulator of viral expression). p12/p13/p30 / R3-G4 are genes encoding accessory proteins. HBZ is a 3’ LTR-directed antisense-transcribed factor important for HTLV-1 oncogenicity. In the region located between ENV and R3/G4, the BLV genome expresses viral microRNAs from internal pol III promoters.
**GENE ACTIVATION THERAPY**

Since several decades, a long debate in the field tried to reconcile apparently conflicting observations. On one side, experimental evidence indicates that the virus is silent mainly because only very few cells expressing viral proteins are detectable in vivo \((12, 13)\). Viral expression can only be detected by very sensitive techniques such as RT-PCR. Furthermore, infected cell clones characterized by a single chromosomal integration site are very stable over time \((14, 15)\). On the other side, there is evidence for active viral expression stimulating a vigorous immune response \((16, 17)\) and for increased turnover of the infected cell population under the control of viral proteins \((18, 19)\). During chronic infection, the host-pathogen interplay thus appears to be characterized by very dynamic kinetics generating equilibrium between viral proliferation and an active immune response \((20)\). In this model, the virus persistently transits between latent and transcriptionally active phases resulting in progressive accumulation of viable infected cells.

We hypothesized that activation of viral expression could create an imbalance in this dynamic equilibrium. However, the net outcome of this type of treatment on infected cell numbers was difficult to predict since induction of Tax expression promotes cell proliferation but also allows destruction of the silent pool by the host immune response. Therefore, the validity of this concept was first tested in the BLV model.

To activate viral expression, we used a histone deacetylase (HDAC) inhibitor, valproic acid (VPA), known to induce hyperacetylation of chromatin. VPA has pleiotropic effects in cells including inhibition of lysine deacetylation \((52)\). Interference with the level of histone acetylation modulates chromatin condensation, which is an essential component of the gene expression pattern. In fact, this process results from an intrinsic balance between the activity of two families of antagonistic enzymes, HDAC and histone acetyltransferases (HAT), respectively removing or incorporating acetyl groups into core histones (Figure 2). Although this model is probably oversimplified, acetyl removal by HDACs restores a positive charge to the lysine residues in the histone N-terminal tails and is thought to increase the affinity of histones for DNA, leading to transcriptional repression. Conversely, impairment of HDAC function with specific inhibitors activates both cellular and viral gene transcription.

As hypothesized, VPA stimulates BLV expression but is concomitantly also pro-apoptotic for leukemic cells in culture \((21)\). VPA treatment of BLV-infected leukemic sheep results in a progressive reduction in the number of neoplastic B lymphocytes. Based on these promising observations in the BLV model, we conducted a single-center, two-year open-label trial, with 19 HAM/TSP volunteers treated with oral VPA. Despite early fluctuations, the proviral loads were not significantly affected after long term VPA treatment \((22, 23)\). The treatment appeared
Figure 2. Chromatin remodeling is regulated by post-translational modifications of histones. HAT (histone acetyltransferases) acetylate chromatin while HDAC (histone deactylases) catalyse the opposite reaction. Decompacted chromatin is generally associated with gene transcription and DNA replication. VPA (valproic acid) inhibits HDACs and affects diverse aspects of cell and viral metabolism.

to be safe as outlined by immunological criteria (i.e. antiviral cytotoxic response). Unfortunately, clinical symptoms of subjects with advanced TSP were not improved. However, this approach might appear to be effective in ATL patients (24).

SYNTHETIC LETALITY

Among viral factors, the Tax protein plays a critical role in pathogenesis. Indeed, Tax stimulates proliferation, compromises genome stability and causes immortalization of primary cells through interaction with host cell proteins (18, 25-27). We recently demonstrated that Tax fires supplementary replication origins and increases DNA replication rates during the synthesis (S) phase of the cell cycle (28). Mechanistically, Tax binds replication origins by interacting with the MCM2-7 helicase (minichromosome maintenance complex), recruits the CBP/p300 acetyltransferase and promotes acetylation of histones at origins of replication. This process allows activation of normally late origins in early S phase and increases DNA replication rates. However, accelerated cell cycle progression compromises genomic stability and generates, in a fraction of the cells, a replicative stress, characterized by the formation of double-strand breaks, activation of DNA Damage Response pathway (DDR)-associated checkpoints and transient or permanent cell cycle arrest.

DDR signaling is orchestrated by the ATM, ATR and DNA-PK checkpoints. These three kinases and their substrate CHK1/CHK2 allow activation of
transcription factors (e.g. NF-kB, p53, AP-1), transiently arrest cell cycle and initiate DNA repair. In case of severe and irreparable damage DDR, signaling drives cells toward a permanent cycle arrest (e.g. apoptosis, senescence, autophagy or necrosis). It is widely accepted that this machinery acts as a barrier against tumorigenesis. However, chronic DDR activation creates a selective pressure that eventually favors outgrowth of malignant clones that are “adapted” to checkpoints owing to genetic or epigenetic defects in DDR signaling. If modulation of replication timing by Tax triggers DDR pathway and cycle arrest, ongoing experiments further reveal that checkpoint adaptation mechanisms occur in the course of HTLV-1 infection and transformation. This process allows cells to proliferate despite the presence of genomic damage and checkpoint activation.

Cells adapted to checkpoints use other repair mechanisms (such as translesion synthesis) to prevent a fatal genetic chaos. DDR aberrations thus render cells dependent on a reduced or alternative set of DNA repair pathways for survival. This feature can be exploited for therapeutic purposes using chemical compounds targeting major components of DNA repair pathway such as caffeine (an inhibitor of ATM / ATR), CGK-733 (inhibitor of ATM / ATR), NU7026 (DNA-PK inhibitor) and UCN-01 (CHK1 inhibitor) (Figure 3). It is noteworthy that drugs targeting some of these pathways are already in clinical trials, sustaining the pertinence of this strategy for human cancer therapy. Alterations of the DNA repair pathways in ATL cells provide therapeutic opportunities working on a principle of synthetic lethality. We indeed postulate that DDR aberrations arising during HTLV-1-mediated transformation render cells reliant on a reduced set of DNA repair pathways for survival. Drugs that inhibit one of these pathways in ATL cells could be useful as single-agent or in combination with current treatments (IFNa, AZT, arsenic trioxide).

![Figure 3. DNA damage initiates several signaling pathways involving kinases ATM (Ataxia telangiectasia mutated), ATR (ATM and Rad3-related) and DNA-PK (DNA-dependent protein kinase). These DNA damage sensors transmit the signal via CHK1 and CHK2 (Cell Cycle Checkpoint Kinases) to the p53 tumor suppressor protein, which in turn regulates cell fate (e.g. cell cycle arrest, DNA repair, senescence or apoptosis). Inhibitors of these pathways (in red) are becoming available.](image-url)
CONCLUSIONS

Understanding two basic mechanisms of HTLV-1 and BLV pathogenesis (i.e. epigenetic control of viral expression and alteration of DNA damage response pathways) has paved the way for novel therapeutic strategies based on the principles of gene activation and synthetic lethality, respectively.

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LIST OF REFERENCES


