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Review

Biology, clinical features, and immunology of human T-cell lymphotropic virus type 2

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Abstract

Human T cell lymphotropic virus type 2 (HTLV-2) is an exogenous retrovirus that establishes a lifelong chronic infection in humans and is rarely associated with disease. HTLV-2 is a common co-pathogen among patients infected with human immunodeficiency virus (HIV). HIV-1/HTLV-2 co-infected individuals have lower plasma HIV-1 levels and delayed rates of $CD4^+$ T cell decline, effects attributed to Tax2, the transactivator protein of HTLV-2. Tax2 has been shown to promote survival and proliferation of T cells, and stimulate cytokine and chemokine production by peripheral blood mononuclear cells and cell subsets. Tax2 activates the canonical pathway of NF-κB, but its role in other signaling pathways has not been clearly elucidated. Herein, we review the most recent information on HTLV-2 biology, clinical features, and infection immunity using data from our research and the related published literature.

Keywords: HTLV-2, Tax2, Rex2, APH-2, innate and acquired immunity

1. Introduction

Human T cell lymphotropic virus type 2 (HTLV-2) is classified in the Retroviridae family, genus Deltaretrovirus and shares 60-70% homology at the nucleotide level with HTLV type 1 (HTLV-1). HTLV-2 binds to $CD8^+$ T cells, its primary target,

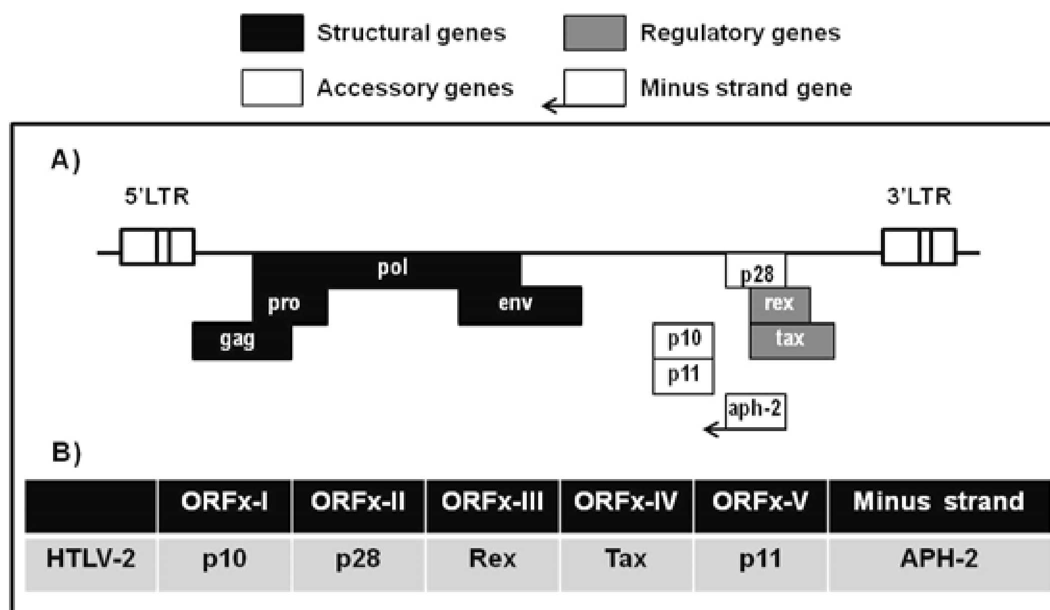
via the glucose receptor Glut-1. After entry, the viral enzymes reverse transcriptase and integrase mediate reverse transcription of viral RNA and integration of the double-stranded DNA copy into the cell genome, respectively. The provirus may remain inactive or be transcribed into progeny virions utilizing the transcription and translation machinery of the host. HTLV-2 is endemic among native Amerindians and pygmy tribes of Central Africa and is prominent among injecting drug users in North America and Europe. The predominant modes of viral transmission occur sexually and parenterally (blood transfusion and contaminated needles in injection drug use), but unlike human immunodeficiency virus type 1 (HIV-1), HTLV-2 is seldom transmitted vertically from mother to child. HIV-1 and HTLV-2 co-infections occur frequently since both viruses share common modes of transmission. HTLV-2 establishes a lifelong chronic infection, which is only rarely associated with any disease. Many features of the immune response to HTLV-2 are unknown, but recent research indicates that Tax2, the HTLV-2 transactivating protein has an important immunoregulatory role during HIV-1 co-infection. A better understanding of HTLV-2 and its regulatory proteins may lead to the development of immunotherapeutic strategies for treatment of HIV-1 infected population.

2. Biology of HTLV-2

2.1. Taxonomy, structure, and genomic organization of HTLV-2

Human T cell lymphotropic virus type 2 also known as human T cell leukemia virus type 2 (HTLV-2) belongs to the Retroviridae family in the genus Deltaretrovirus; other members of this family include HTLV types 1, 3, and 4, bovine leukemia virus (BLV), and simian T-cell leukemia virus (STLV) types 1, 2 and 3 [1, 2]. HTLVs are enveloped viruses with an electron-dense centrally located core with a diameter of approximately 100-120 nm. The virions contain two positive-sense, covalently bound single-stranded RNAs (ssRNA) that are complexed with the viral enzymes reverse transcriptase (RT), integrase (IN), and protease (PR), and surrounded by capsid (CA) proteins. The outer part of the virion consists of a membrane-associated matrix protein (MA) and a lipid bilayer studded with the viral envelope proteins [3]. As a retrovirus, the structure of HTLV-2 is provided mainly by its MA protein, which is structurally homologous to that of HIV-1, suggesting that this structure is evolutionarily conserved [4]. The primary structural elements are comprised of the MA, CA, and nucleocapsid (NC) proteins [3].

The HTLV-2 genome (*LTR-gag-pro-pol-env-X-LTR*) has been completely sequenced, and consists of a linear genome of single stranded RNA with 8,952 nucleotides that has a GC content of 53.8% (Figure 1). The genome contains structural genes (*gag, pol,* and *env*), regulatory genes (*tax* and *rex*), and accessory genes (*p10, p11, p28,* and the recently identified HTLV-2 antisense gene *aph-2*) [5]. Transcription from the 5'LTR promoter generates three major size classes of mRNAs including a 9kb full-length genomic mRNA coding for Gag-Pro-Pol, a 4kb singly-spliced mRNAs coding for the envelope glycoprotein (Env), and mRNAs of approximately 2kb encoding proteins of the X region. The *pro* gene encodes a protease that cleaves the precursor Gag polyprotein encoded by *gag* into the structural proteins of the virion (NC, MA, and CA). The *pol* gene encodes 982 amino acids (aa) for the reverse transcriptase and integrase enzymes and the *env* gene encodes 486 aa for the envelope polyprotein that also is cleaved into the functional surface (SU) and Env proteins. The X region of the provirus encodes for accessory proteins responsible for regulation of viral protein expression and replication [6]. HTLV-2 has 60-70% homology to HTLV-1 in highly conserved regions such as the *gag, pol, env, tax,* and *rex* genes, with lesser homology in the long terminal repeats (LTR), protease, and the pX region [7]. The X region of HTLV-2 encodes five major open reading frames (ORFs, x-I to x-V). The x-III and x-IV ORFs code for the essential regulatory proteins Rex and Tax, respectively, and are produced from a bicistronic doubly-spliced mRNA containing exons 1, 2, and 3. ORFx-II produces the singly-spliced p28, the truncated isoform of Rex (tRex) mRNAs and doubly-spliced mRNAs that code for the accessory proteins p10 and p11 from x-I and x-V ORFs, respectively [8, 9] (Figure 1). HTLV-2, like HTLV-1, contains a promoter located on the



	ORFx-I	ORFx-II	ORFx-III	ORFx-IV	ORFx-V	Minus strand
HTLV-2	p10	p28	Rex	Tax	p11	APH-2

Figure 1. Structure of the HTLV-2 genome. **A)** The scheme shows the HTLV-2 proviral genome with the long terminal repeats (LTRs) denoted as boxes in the farthest opposite sides. Structural (*pol, env, gag*, in black), regulatory (*tax, rex*, in gray) and accessory (*p28, p10, p11*, in white) genes are transcribed by promoters in the 5'LTR. The minus strand (*aph-2*, with arrow) gene is transcribed by a promoter in the 3'LTR. **B)** The X regions of HTLV-2 encode five major open reading frames (ORFs) termed x-I through x-V coding for regulatory and accessory proteins.

negative sense strand within the 3'-LTR that is used to encode the antisense protein of HTLV-2 (APH-2) [5, 10]. Currently, only one singly-spliced *aph-2* mRNA has been identified in HTLV-2 infected cells and its transcription is initiated within the 3′ LTR (U5 and R regions) at multiple positions [5]. A similarly located promoter directs transcription of unspliced and multiple singly spliced variants of HTLV-1 b-ZIP (*hbz*) antisense gene [11, 12].

2.2. Lifecycle of HTLV-2

While the details of the lifecycle of HTLV-2 have not been well-characterized, it appears to follow the general lifecycle of other human and primate retroviruses. HTLV-2 infects host cells as a classic retrovirus does, attaching itself to a host cell receptor. HTLV-2 binds to $CD8^+$ T cells, its primary target, via the glucose receptor Glut-1 [13], which is highly expressed in these cells. $CD4^+$ T cells, the primary target of HTLV-1, express high levels of heparan sulfate proteoglycans (HSPGs), which are required for efficient entry of HTLV-1. Transfection studies revealed that over-expression of Glut-1 in $CD4^+$ T cells increases HTLV-2 entry, while expression of HSPGs on $CD8^+$ T cells increases entry of HTLV-1, demonstrating that HTLV-1 and HTLV-2 have different receptor requirements for T-cell entry [14]. Efficient HTLV entry into the host cell usually requires direct cell-cell interaction [15], although successful *in vitro* infections with cell-free virus particles have been reported in several cell lines [15, 16]. After entry, the viral enzymes RT and IN mediate reverse transcription of viral ssRNA into double-stranded linear DNA (dsDNA), which then enters the nucleus and integrates into the host cell genome. Following integration, the provirus may remain inactive or is transcribed into progeny virions. Host cell gene transcription and protein synthesis machinery are used to complete the processes of viral gene expression, protein production, and assembly.

In order to achieve successful transcription of the DNA provirus to viral RNA, the expression of early gene products is necessary. The first genes to be expressed are *tax* (transcriptional activation protein) and *rex* (post-transcriptional regulator protein involved in transport and expression of the

messenger RNAs for the viral structural proteins) [17]. Once the Rex protein is made, it down-regulates Tax and its own expression, with subsequent synthesis of the other viral proteins. The host cell machinery and cellular factors permit rapid transcription of the DNA provirus into viral RNA within the nucleus, which then translocates to the cytoplasm. Viral assembly occurs at the cell membrane with viral proteins that have been formed by translation of unspliced or singly spliced messenger RNAs in the cytoplasm. The viral polyproteins are processed into functional subunits via cleavage by viral and cellular proteases. After assembly, budding and release from the host cell, the newly formed virions are capable of infecting other cells and completing the replication cycle of the virus [18]. Although the expression, functional properties of the viral genetic determinants, and lifecycle of HTLV-2 and HTLV-1 are remarkably similar, their pathogenic potentials are significantly distinct. Recent studies indicate that the differences in properties and functions of accessory and regulatory proteins expressed from the pX region of the virus are critical for the pathological differences between the HTLVs [9].

2.3. Regulatory proteins of HTLV-2 (Tax2 and Rex2)

HTLV-2 and HTLV-1 have two major transcriptional regulators, Tax and Rex. Tax2 is the major transcriptional regulator of HTLV-2, activating the HTLV promoter via three imperfect 21 nucleotide repeats referred to as the Tax response element (TRE) found within U3 region in 5'LTR [19]. Tax2 is required for viral replication and enhances the provirus expression during the early phase of infection. Tax2 has been characterized mainly from HTLV-2 subtypes A and B [20]. Tax2B has 356 aa residues, whereas Tax2A has a 25 aa C-terminal truncation (331 aa). Tax1 (40 kDa protein) encompasses 353 aa and is highly conserved among all HTLV-1 serotypes. Tax1 and Tax2 are 85% conserved at the amino acid level and have several common domains in the N-terminal region including a cyclic AMP response element-binding protein (CREB) and a zinc-finger domain (Figure 2) [21, 22]. Structural and functional similarities in the Tax1 and Tax2 C-terminal regions comprise an ATF/CREB-activating domain, nuclear localization signal (NLS), and nuclear export signal (NES) [23, 24]. Tax1, not Tax2, includes two leucine zipper-like regions (LZR) that are necessary for its DNA interaction and for protein dimerization, as well as binding domains for proteins involved in chromatin remodeling, cell cycle control, NF-κB2 activation, and p300 binding, all of which are absent in Tax2 [25, 26]. Both Tax1 and Tax2 proteins constitutively activate the canonical NF-κB pathway by interacting with RelA and the IκB kinase complex (e.g., IKKα, IKKβ, and NEMO/IKKγ) [27]. Tax1 has various post-translational modification (PTM) sites for phosphorylation, ubiquitination, and small ubiquitin-like modifier (SUMO)ylation [9]. The main structural difference between Tax1 and Tax2 is represented by the lack of a leucine zipper region (LZR) in Tax2, including a region responsible for non-canonical NF-κB activation [28-30] and of the C-terminal motif, which mediates association with proteins containing PDZ domains [29]. Compared to Tax1, Tax2 shows lower overall

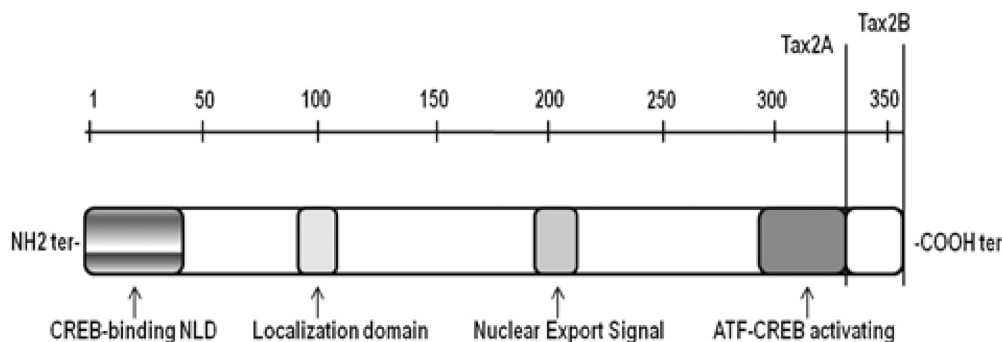


Figure 2. Structural and functional domains of Tax2. Tax2A has 331 aa and Tax2B has 356 aa.

activity with respect to transactivation, transformation capacity, and inhibition of p53 functions [31]. In contrast to Tax1, Tax2 is unable to induce micronuclei or does so inefficiently [32], does not perturb development and maturation of pluripotent hematopoietic progenitor cells [33], and does not induce G0/G1 cell cycle arrest [34].

Rex2 protein (26 kDa) is the major post-transcriptional regulator of HTLV-2 containing 170 aa and sharing 60% homology with Rex1 (from HTLV-1) at the amino acid level with overlapping major functional domains, the RNA binding domain (RBD)/NLS, the core activation domain (AD)/NES, and multimerization domains [35-40]. Rex localizes in the nucleus, nucleoli, and nucleolar speckles [37-40]. Although mainly detected in the nuclear compartment, Rex actively shuttles between the nucleus and the cytoplasm [39], a property that is linked to its ability to transport incompletely-spliced viral RNA from the nucleus to the cytoplasm, which prevents further splicing and promotes efficient translation of the structural proteins [39]. Both Rex1 and Rex2 phosphoproteins bind to mRNAs in the Rex responsive element (RxRE1 and RxRE2, respectively). Within the mRNA, RxRE-1 is a stem-loop structure of 205 nucleotides that is found in the 3′ LTR (U3/R region), but has subsequently been shown to be present, at least in part, in the 5′ LTR. The RxRE-2 is 226 nucleotides in length and is located within the 5′LTR and maps to the U5/R region [40]. Rex1, but not Rex2, is capable of transporting all viral mRNAs including *tax/rex* mRNA from the nucleus to the cytoplasm and it enhances the expression of Tax to promote transactivation of LTR. Therefore, Rex1 may have a stronger effect on viral replication through the enhancement of Tax1 expression compared to Rex2. The different roles of Tax and Rex may contribute to the differences in the pathogenesis observed between HTLV-1 and HTLV-2 [39, 40].

2.4. Accessory proteins of HTLV-2 (p28, p10, p11, APH-2)

The accessory proteins of HTLV-2 are not required for immortalization of cells *in vitro,* but studies have demonstrated their importance *in vivo* [41, 42]. The p28 protein of ORF II functions in a post-transcriptional manner to retain *tax/rex* mRNA in the nucleus, thus reducing viral expression, an effect that may result in viral latency and blunting of the immune recognition of infected cells [43]. This function is homologous to p30 of HTLV-1; however, p28 lacks the transcriptional repression ability of p30 since it is unable to interact with CBP/p300. In analogy to HTLV-1 p12, HTLV-2 p10 and p11 were shown to bind the major histocompatibility complex (MHC) heavy chain; however, p10 and p11 did not bind other targets of p12, such as the IL-2R β-chain or the 16-kDa subunit of the vacuolar H+ ATPase [42].

The most recently discovered accessory gene of HTLV-2 is *aph-2*. APH-2 (antisense protein of HTLV-2) is a 183 aa protein that is localized mainly in the nucleus. The aph-2 mRNA has been detected in HTLV-2-infected cell lines and in HTLV-2-infected patients [10]. Like HBZ of HTLV-1, APH-2 binds to CREB and prevents its interaction with Tax, thereby repressing Tax-mediated transcription [44]. Since experimental evidence has suggested a role for HBZ in HTLV-1-associated disease, laboratories are starting to perform comparative studies between HBZ and APH-2. Marban *et al.* [45] and unpublished data from Dr. Green's research group have demonstrated that unlike HBZ, APH-2 stimulates basal AP-1 transcription by interacting with c-Jun and JunB through its non-conventional bZIP domain. Interestingly, when Tax2 and APH-2 are co-expressed, APH-2 acts as an inhibitor of Tax2-mediated activation of AP-1 transcription instead of acting in a synergistic manner [45].

3. HTLV-2 Clinical Features

3.1. Transmission, detection, and epidemiology

HTLV-1 is transmitted vertically from mother to child through childbirth or breastfeeding. Vertical transmission appears to be a much less frequent mode of transmission for HTLV-2. However, HTLV-1 and HTLV-2 are similarly transmitted from person to person via sexual contact (primarily male to female), and parenterally through contaminated blood including transfusion of blood from an infected donor or by needle sharing among intravenous drug users (IDUs) [46, 47]. It is estimated that approximately 15-20 million people are infected with either HTLV-1 or HTLV-2 worldwide [47, 48].

The United States initiated screening of donated blood for HTLV-1 infection in 1988 and for HTLV-2 infection in 1997 via enzyme-linked immunosorbent assay (ELISA), which is highly specific and sensitive for both virus types. Testing has effectively prevented HTLV transmission through blood and its components, leaving a remaining risk of transmitting HTLV infection by screened blood of $1/6.41 \times 10^5$ units [49]. After screening was implemented, the incidence rate of HTLV in blood donors was determined to be $1.59/1 \times 10^5$ person-years [50]. Currently, screening includes detection by ELISA followed by a confirmatory Western blot (WB) [51]. WB detection uses recombinant HTLV-1 Env glycoproteins and Gag proteins that are incorporated into WB strips. HTLV-1 seropositive results are defined by the presence of antibodies against either gp46 or gp62/68 (both Env protein bands) and p19, p24, or p53 (one of the Gag bands). HTLV-2 seropositivity is confirmed by the presence of rgp46-2. Polymerase chain reaction (PCR) detection of HTLV-1 and HTLV-2 is available primarily through reference laboratories. For research purposes, quantitative PCR can be used to determine the number of HTLV-1 or HTLV-2 DNA copies per fixed number of peripheral blood mononuclear cells (PBMCs). PCR quantification also is required in infants who may have false-positive results because of circulating maternal anti-HTLV antibodies. Patients diagnosed with HTLV-1 or HTLV-2 infection should undergo screening for HIV-1 because of the common modes of viral transmission [50].

HTLV-2 is endemic among highly separated and often geographically isolated groups, including Amerindian populations throughout North, Central, and South America, and in Pygmy tribes in Central Africa [52-54]. It has been theorized that HTLV-2-infected Asian population migration into the New World through the Behring Land Bridge introduced HTLV-2 into the American continent [55]. In the United States, HTLV-2 infection among Amerindians may approach a seroprevalence rate of 13% in some tribes. Subsequently the virus may have been transmitted from indigenous people to IDUs resulting in epidemic spread of the virus [56-58]. Highest rates of HTLV-2 appear among African American IDUs, with a seroprevalence of approximately 20% [59].

3.2. Discovery, clinical characteristics, and associated diseases

HTLV-2 was first discovered in 1982 in a patient with a variant form of hairy-cell leukemia, a rare type of leukemia [60]; however, this may have been an unusual event. Due to the low viral replication of HTLV-2 there are usually no clinical symptoms, and the acute form of HTLV-2 infection is rarely suspected or diagnosed. The detection of HTLV-2 infection primarily results from blood donation testing performed because of a familial history with the infection, or because the individual was infected with HIV-1. At present, HTLV-2 has not been established to be the etiologic agent of any specific disease [47], while some associations have been proposed including neurological illness and inflammatory diseases [59, 61, 62]. Recently it was reported that HTLV-2 infection was linked with higher lymphocyte and platelet counts, but it has not been associated with oncogenesis [63]. Conversely, approximately 3-5% of HTLV-1 carriers develop clinical manifestations, including adult T-cell leukemia/lymphoma (ATLL), HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and other autoimmune and inflammatory diseases (reviewed in [64, 65]).

3.3. HTLV/HIV co-infections

HTLV-1 and HTLV-2 are common co-pathogens among HIV-infected individuals mostly due to the similar modes of virus transmission [66-69]. HIV-1/HTLV-1 co-infections are more frequently reported in South America, the Caribbean, and Africa [70-72]; co-infected patients have increased risk for development of HAM/TSP [73]. Studies have suggested that HTLV-1 alters the natural history of HIV-1 infection inducing a faster clinical progression and a shorter survival time [74]. HIV-1/HTLV-2 co-infections predominate in the United States and Europe [66-69]. The prevalence of HTLV-2 infection among HIV-infected IDUs is approximately 10% in large metropolitan areas in the United States [75]. Interestingly, cohort studies from our group and others have found that HTLV-2 infection exerts a protective role in the progression of HIV-1 disease in this co-infected population [76, 77]. Lower plasma HIV-1-RNA levels were also found

in HIV-1/HTLV-2-co-infected patients compared to HIV-mono-infected individuals [76-78]. In addition, the proportion of long-term non-progressors (LTNPs) to AIDS in the HIV-1/HTLV-2 co-infected population (13.5%) was significantly higher than in HIV-1 mono-infected cases (1.1%). The LTNP phenotype with CD4 counts >500 cells/μl and a stable HIV viremia between 1000-1500 copies/ml did not develop opportunistic infections or require antiretroviral therapy [77]. The protective role for co-infection by HTLV-2 on HIV-1 disease progression, observed by various clinical studies and supported by several laboratory evidences, has been hypothesized to be the result of maintaining normal-range levels of CD4 and CD8 counts, lowering HIV replication, and activating the immune system [74].

The CC-chemokines MIP-1α, MIP-1β, and RANTES, produced by activation of macrophages (MØ), dendritic cells (DC), T cells, natural killer (NK) cells, and gamma delta (γδ) T cells, block the CCR5 co-receptor preventing HIV infection *in vitro* [79, 80] or during simian immunodeficiency virus (SIV) infection *in vivo* [81]. Macaques immunized with recombinant SIVgp120 and p27 in alum had up-regulated levels of CC-chemokines that inversely correlated with down-modulation of CCR5 and low plasma SIV mRNA levels [82]. CCL3L1 (an isoform of CCL3), preferentially induced in HTLV-2 exposed seronegative HIV individuals and in LTNP/HTLV-2/HIV-1 co-infected persons [83], acts as a potent effector against both HIV infection and disease progression [84].

Recently, it was reported that Tax2 induces the production of high levels of the antiviral CC-chemokines MIP-1α, MIP-1β, and RANTES by PBMCs and monocyte derived macrophages (MDMs) [85, 86] with the concomitant down-regulation of CCR5 expression on lymphocytes [85]. Interestingly Tax2 protein also was shown to mediate inhibition of HIV-1 replication in PBMC cultures *in vitro* [87]. These findings follow a paradigm observed with other viral co-pathogens infecting persons with HIV-1. Studies showed that co-infection with GB virus C (GBV-C), formerly known as hepatitis G virus (HGV), slowed HIV-1 disease progression and co-infected individuals often survive longer than those without GBV-C [88]. African variants of GBV-C have been shown to replicate in PBMCs and to inhibit the replication of HIV *in vitro* via the mediation of induced CC-chemokines and the down-regulation of CCR5 [89, 90]. Another group has reported *in vivo* and *in vitro* evidence of the inhibition of HIV-1 replication by measles virus (MV) co-infection concurrent with intense immune activation including higher plasma levels of RANTES in HIV-infected children with measles [91, 92].

4. Immunology of HTLV-2

4.1. Cells infected by HTLV-2 and innate immunity

Innate immunity involves transient and non-specific mechanisms of host defense, thereby providing immediate defense against infection by pathogens. The recruitment of immune cells to sites of infection through chemokines, complement cascade activation, removal of foreign substances by blood white cells, activation of the adaptive immune system, and providing a physical-chemical barrier to infectious agents are the major functions of the innate immune system. Innate immunity plays a critical role in the primary host response to viral infections. *In vivo*, HTLV-2 and HTLV-1 not only infect T cells that participate in adaptive immunity, but also other cells that contribute to innate immunity [93]. HTLV-2 provirus is found predominantly in $CD8^+$ T lymphocytes of infected individuals, although infection in $CD4^+$ T cells, the major target of HTLV-1, also has been reported [94, 95]. In addition, NK cells were found to be naturally infected by HTLV-2, and they might constitute a major reservoir of infection [96]. HTLV-2-infected-NK cells may be functionally impaired, resulting in viral escape from innate immune surveillance [96]. Conversely, HTLV-1 can infect lymphocytes, monocytes, blood or monocyte-derived dendritic cells (moDC), and plasmacytoid DC (pDC) *in vitro*. $CD56^+$ NK cells have been reported to spontaneously proliferate *in vitro*, positively correlating with HTLV-1 proviral load, but not in association with HAM/TSP [97].

Members of the CC-chemokine family play a major role in innate immune responses against viral infections. It has been hypothesized that

HTLV-2 infection of lymphocytes and MØs could provide a source of chemokines that result in an antiviral response against HIV-1 infection [79, 98]. CC-chemokines have been correlated with innate resistance to HIV-1 infection [99], decreased viral loads in infected individuals [100], and protection against disease progression to AIDS [101, 102]. HTLV-1 and HTLV-2 have fairly broad cellular tropisms, including both $CD4^+$ and $CD8^+$ T cell subsets, DC, monocytes, and MØ. *In vitro* studies from our group have examined the effect of HTLV-2 Tax (e.g. recombinant Tax2 protein and Tax2 expressed via a recombinant adenoviral-vector) on CC-chemokine expression in PBMCs and in isolated cell subsets including MDMs, $CD4^+$ T cells, $CD8^+$ T cells, and NK cells. It was shown that Tax2 induces the production of high levels of the antiviral CC-chemokines MIP-1α, MIP-1β, and RANTES by PBMCs and MDMs [85, 86]. Previous work by Lewis *et al.* [103] and others have implicated $CD8^+$ T cells as a potential source of CC-chemokines in HTLV-2-infected individuals. Thus, we compared production of CC-chemokines in Tax2-treated $CD4^+$ and $CD8^+$ T cell subsets and other cellular compartments. $CD4^+$ (96%) and $CD8^+$ (97%) T cells were purified using magnetic bead separation. The data showed significantly higher numbers of $CD4^+$ and $CD8^+$ T viable cells when treated with Tax2 protein ($p < 0.05$) as compared to mock-treated controls. $CD4^+$, but not $CD8^+$ T cells, showed significantly increased release of chemokines over mock-treated-cells ($p < 0.05$) (Figure 3). These results were unexpected since HTLV-2 preferentially infects $CD8^+$ T cells *in vivo* and *in vitro*. The data support the hypothesis that HTLV-2 infection of $CD8^+$ T cells could induce CC-chemokine expression in bystander cells, including helper T lymphocytes, either via cell-cell contact, or perhaps through secretion of Tax2 into the extracellular microenvironment.

4.2. HTLV-2 acquired immunity

The acquired immune response is a delayed but specific response allowing the development of immune memory. Many features of the immune response to HTLV-2 are poorly understood due to paucity of information currently available. Some reports have indicated that HTLV-2 results in activation and spontaneous proliferation of T cells, as well as other events leading to production of various cytokines and chemokines [83, 103-105]. Analysis of immunological and virological parameters in HIV-1/HTLV-2 co-infected patients and a control group of HIV-mono-infected subjects showed lower plasma HIV-RNA levels in co-infected individuals than in HIV-mono-infected patients, despite the two groups having similar $CD4^+$ T cell counts. Co-infected patients also had significantly lower levels of CD38 expression (marker of cell activation) in total $CD8^+$ T cells and in its naive subset [105]. CCL4 positive/IFN-γ negative cells were the main contributors to HIV Gag-specific responses in co-infected patients, whereas the response was dominated by CCL4 positive/IFN-γ positive cells for HIV-mono-infected subjects. This finding indicated that HTLV-2 co-infection may exert a protective role on HIV disease progression by lowering HIV replication and immune activation. A predominance of CCL4 single-positive HIV-specific $CD8^+$ T cells in HIV-1/HTLV-2 co-infected patients could explain this effect [105].

HTLV-2 has been shown to induce the production of granulocyte macrophage colony stimulating factor (GM-CSF), IFN-γ, and stem cell factor (SCF) in $CD34^+$ TF-1 cells through activation of the STAT signaling pathway [104]. IL-6, TNF-α, and CC-chemokines (MIP-1α, MIP-1β, and RANTES) were secreted from cultured PBMCs of HTLV-2 and HIV-1 co-infected individuals, indicating that HTLV-2 can influence HIV replication, *in vitro*, via up-regulation of these CC-chemokines [105]. The cell activation induced by HTLV-2 also induces the spontaneous expression of GM-CSF, IFN-γ, which are cytokines with ability to down-regulate CCR5 receptor expression, and CCL3L1, an isoform of CCL3 that concurrently down-regulates the expression of CCR5 [83].

Antiviral $CD4^+$ T cell responses are of central importance in driving B cell and $CD8^+$ T cell responses *in vivo*. The immune response to immunodominant envelope epitopes of HTLV-2 (K-55/162-205) and HTLV-1 (MTA-1/162-209) were assayed in HTLV-2 and -1 infected individuals from diverse geographic areas. By synthetic

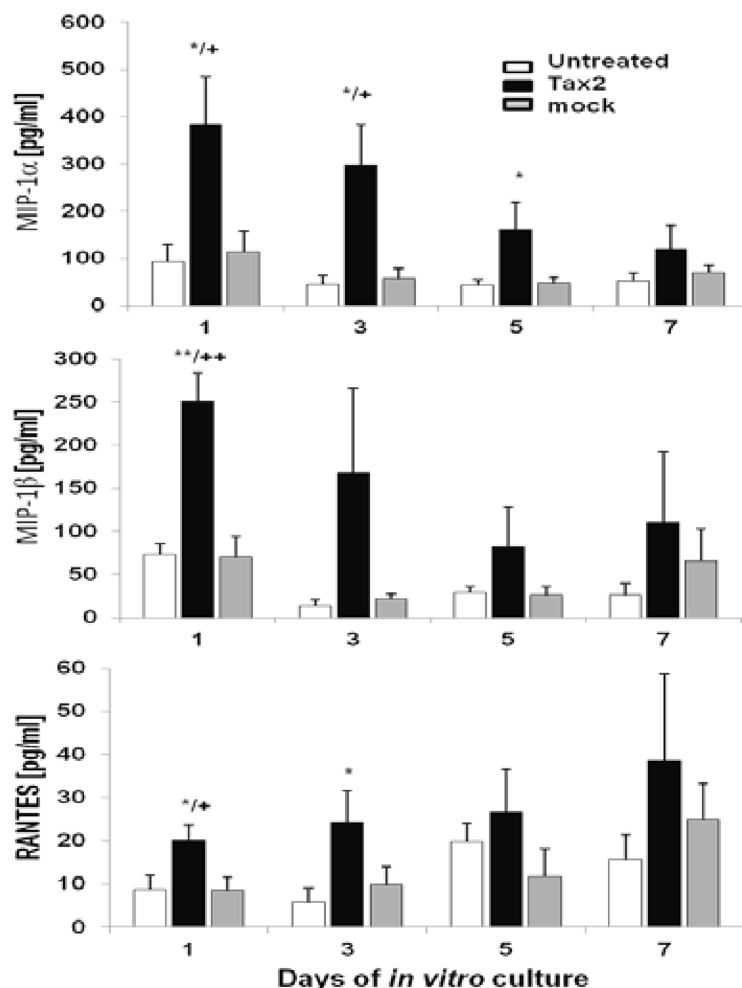


Figure 3. CC-chemokine levels (MIP-1α, MIP-1β, and RANTES) detected in the supernatants of $CD4^+$ T cells treated with Tax2 protein [100 pM]. *$p < 0.05$, **$p < 0.01$, vs. untreated; $+p < 0.05$, $++p < 0.01$; vs. mock-control.

peptide-based serologic typing, all of these specimens could be typed as HTLV-1 or -2 providing indirect evidence for the conservation of immunodominant HTLV Env epitopes in diverse geographic populations [106].

4.3. Tax2 transactivation of cellular genes involved in immunity

HTLV-1 Tax up-regulates the expression of genes encoding cytokines, chemokines, cell surface ligands, and their receptors, in a NF-κB, AP-1, CREB/ATF and/or NFAT dependent manner. These include the IL-2Rα-chain, IL-9, IL-13, IL-15/IL-15R, IL-21/IL-21R, IL-8, CCL2, CCL5, CCL22, CCR9, CXCR7, CD40, OX40/OX40L, and 4-1BB/4-1BBL [107-117]. Transient transfection studies showed that Tax1 induces the expression of IL-2 through the transcription factor NFAT in Jurkat cells treated with either TPA or ionomycin [118]. IL-2 and IL-2R α-chain are crucially important for T-cell immortalization by Tax1 since the immortalized cells are dependent on IL-2 for their growth. HTLV-1 Tax transactivates a variety of cellular genes through the NF-κB pathway including IL-2, IL-2Rα, GM-CSF, TGF-β, TNF-β, c-*myc*, vimentin, OX40L, IL-6, IL-8, IL-15, and VCAM-1 [119]. In contrast, Tax2 is a less potent activator of the NF-κB pathway [120]. Tax2 interacts with different components of the canonical pathway (IKKγ/NEMO, the related proteins RP/Optineurin, p65 [121], and the adaptor protein TAB2 [122], but does not recognize p100 [120], which is a factor of the non-canonical pathway).

The spontaneous production of CC-chemokines has been linked to the transactivation of CCL4 and CCL5 gene promoters by Tax2 [103]. We recently reported that extracellular Tax2 proteins induce the expression of CC-chemokines from PBMCs and MDMs [85, 86]. These antiviral chemokines modulate CCR5 HIV-1 co-receptor expression and may consequently influence HIV-1 pathogenesis [87]. Interestingly, Tax2 expressed via an adenovirus vector (Ad-Tax2) was found to induce secretion of high levels of these CC-chemokines by MDMs [86]. A recent study assessing the role of Tax2-mediated activation of the NF-κB signaling pathway on the production of the antiviral CC-chemokines MIP-1α, MIP-1β, and RANTES tested recombinant Tax2 protein or Ad-Tax2 for their ability to activate the NF-κB pathway in cultured PBMCs in the presence or absence of NF-κB pathway inhibitors (PDTC or NF-κB super-repressor). Significant release of the antiviral chemokines by PBMCs was shown after the activation of p65/RelA and p50, subunits of canonical NF-κB signaling. The secretion of these molecules was significantly reduced ($p < 0.05$) by both inhibitors indicating that Tax2 promoted innate antiviral immune responses primarily through the activation of the canonical NF-κB pathway [123].

In addition to inducing the chemokines MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5 [85, 86], PBMCs treated with Tax2 (recombinant Tax2 or transduced with Ad-Tax2) induced the production of MDC/CCL22, Gro-α/CCL1, and IL-8/CCL8 in PBMCs. In HIV-1/HTLV-2 co-infected individuals, the production of these chemokines by Tax2 may play a role in HIV-1 disease. In fact, IL-8 was reported to decrease HIV-1 transcription in both lymphocytes and ectocervical tissue explants; the decrease in viral RNA expression was associated with reduced HIV-1 replication [124]. In addition, MDC/CCL22 was reported to have HIV-suppressive effects among antiretroviral-treated children; this study showed a significant direct relationship between percent of $CD4^{+}$ T cells and the production of both CCL3 and CCL22 chemokines [125].

High levels of IL-8, IL-6, and TNF-α, major mediators of the inflammatory response, were released in the cell-free cultures from Tax2-treated-PBMCs; the anti-inflammatory cytokine IL-10 also was highly expressed in the supernatants of cultured PBMCs treated with Tax2 (Figure 4). Antiviral responses must be tightly regulated to rapidly defend against infection while minimizing inflammatory damage [126]. The observed cytokine profile may be involved in activation, co-stimulation, and differentiation of T cells and monocytes during HIV-1/HTLV-2 co-infections. These results mirror those reported for endothelial cells co-cultured with HTLV-1 [127]. Similarly, Banerjee *et al.* showed that HTLV-1 infection in astrogliomas resulted in a prominent induction of IL-1β, IL-1α, TNF-α, TNF-β, and IL-6 expression. Tax1 or Tax2, expressed in lentiviral vectors used to transduce primary human astrocytomas and oligodendrogliomas, also induced the transcription of these cellular genes [128].

High levels of Th17-related cytokines including IL-6, TNF-α, TGF-β1, and G-CSF were observed in Tax2-treated cells. Furthermore, IL-6 and TNF-α have been detected in T cells from HTLV-1 and HTLV-2 patients [129]. In contrast, HIV-infected individuals have shown severe loss of Th17 cells during infection, which has been linked to increased plasma viremia [130], and a gradual decline in Tregs during disease progression, associated with increased immune activation [131]. These data suggest that the expression of Th17-related cytokines may be of interest, given the potential role of Tax2 to induce the differentiation and maintenance of the Th17 cell population in HIV-1/HTLV-2 patients. HBZ of HTLV-1 has been demonstrated to support the differentiation of induced T-regulatory cells (iTregs) by enhancing TGF-β expression. It was discovered that HBZ strengthened the interaction of the transcription factor p300 and Smad3, inducing the expression of Smad-dependent genes such as Foxp3 [132]. Luciferase reporter assays were utilized to examine if APH-2 was able to enhance TGF-β signaling. We found that in contrast to HBZ, APH-2 had a slight but statistically significant inhibition of TGF-β signaling (Figure 5). It is important to note that the reporter used was specific for Smad3, which along with Smad2 and Smad4 are important for iTreg diffentiation, whereas only Smad2 is important for Th17 differentiation [133]. These data suggest that upon TGF-β induction, APH-2 could possibly free-up

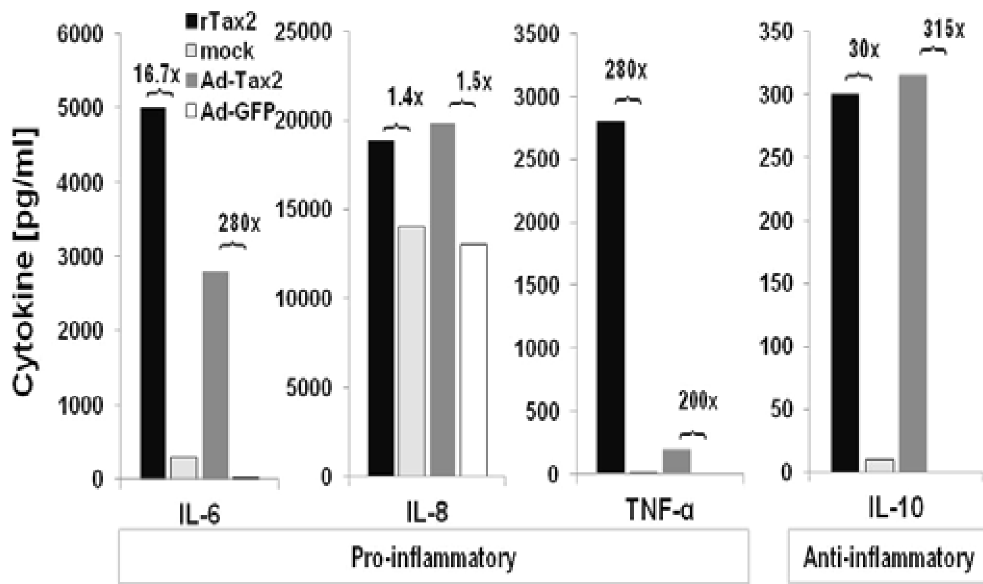


Figure 4. Pro- (IL-6, IL-8, TNF-α) and anti-inflammatory (IL-10) cytokine levels assessed from Tax2-treated-PBMC supernatants. Cell-free supernatants from Tax2-treated-PBMCs (rTax2 or expressed in adenoviral vectors) were tested by multi-analyte ELISA.

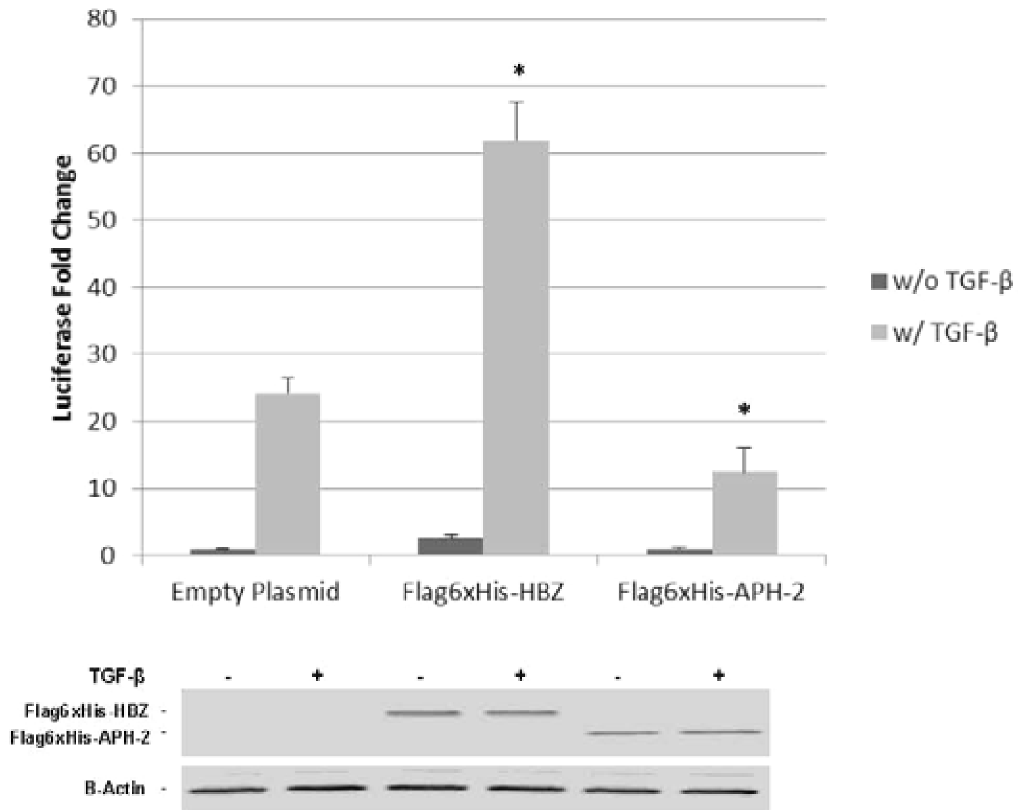


Figure 5. Luciferase levels from a 9xCAGA-reporter construct of HepG2 cells transfected with HBZ and APH-2 and treated with TGF-β. *$p < 0.01$ vs. empty plasmid w/ TGF-β.

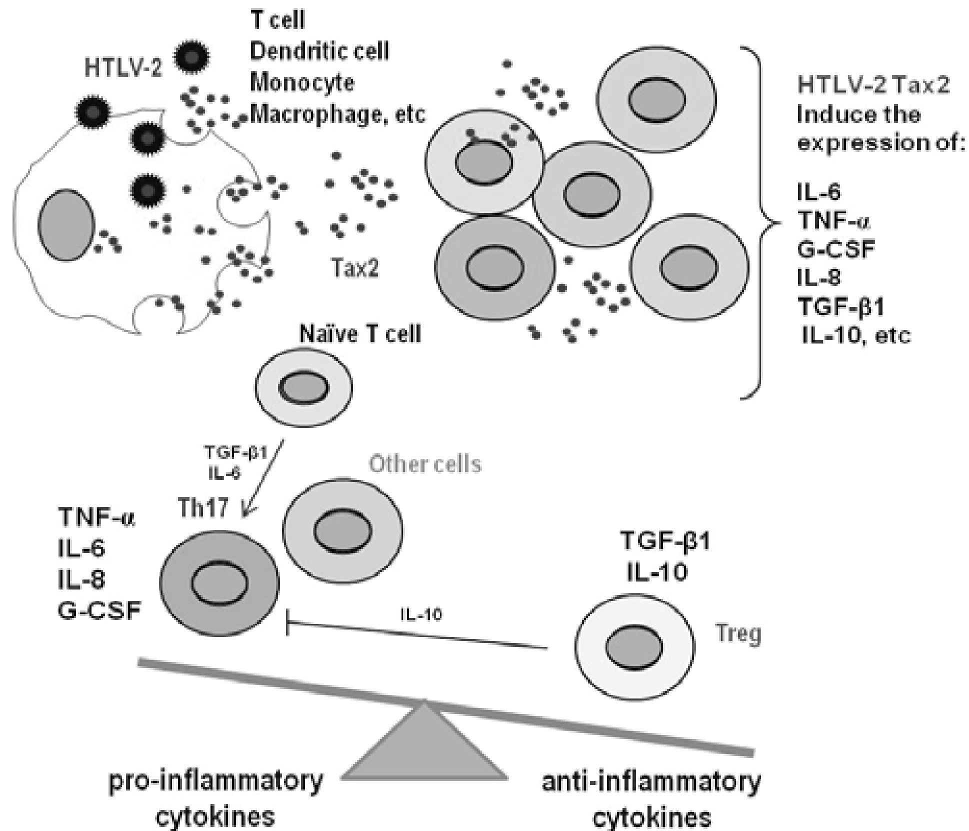


Figure 6. Proposed model of Tax2 in the induction of immune responses. HTLV-2 Tax induces the expression of potent pro-inflammatory cytokines (e.g. IL-6, IL-8, TNF-α) by T cells, monocytes, macrophages, etc. Tax2 also induces the production of the anti-inflammatory cytokine IL-10. Tax2 may maintain a profile favoring pro-inflammatory cytokine expression that could help to inhibit HIV-1 virus replication.

Smad2 and help the subset of infected $CD4^+$ T cells differentiate into Th17 cells instead of Tregs. The balance between pro-inflammatory Th17 and immunoregulatory T cells may be critical in HIV-1 pathogenesis [126], and may have a protective role against HIV-1 progression in the HIV-1/HTLV-2 co-infected population (Figure 6).

Conclusion

Since HTLV-2 is not clearly linked with any disease, the virus has received little attention except for the interest generated by its particularly high prevalence among IDUs. It is very interesting that HTLV-2, a virus that infects IDUs and their sexual partners at very high rates, results in a lifelong and persistent infection that is largely silent and asymptomatic. Yet, the virus expresses Tax2, a protein critical for the lifecycle of HTLV-2, which also appears to be a potent immunomodulatory protein. In scenarios where HTLV-2 expression is up-regulated, such as during co-infection with HIV-1, the potential for Tax2 to modulate immune responses is plausible and intriguing. In contrast to HTLV-1, HTLV-2 lacks the potential to cause either ATLL or HAM/TSP. Therefore, the continuing investigations of the mechanism of how HTLV-2 and Tax2 modulates innate immune responses will have enormous benefits among the HIV-1/HTLV-2 co-infected population and will further assist in the development of antiretroviral drug therapy strategies for treatment of HIV-1 infected individuals.

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Conflict of interest statement

There are no conflicts of interest.

References

1. Vandamme, A-M., Salemi, M. and Desmyter, J. 1998, Trends. Microbiol., 6, 477.
2. Matsuoka, M. and Jeang, K. T. 2007, Nat. Rev. Cancer, 7, 270.
3. IARC. 2012, IARC Monogr. Eval. Carcinog. Risks Hum., 100B, 315.
4. Christensen, A. M., Massiah, M. A., Turner, B. G., Sundquist, W. I. and Summers, M. F. 1996, J. Mol. Biol., 264, 1117.
5. Halin, M., Douceron, E., Clerc, I., Journo, C., Ko, N. L., Landry, S., Murphy, E. L., Gessain A., Lemasson I., Mesnard J. M., Barbeau B. and Mahieux, R. 2009, Blood, 114, 2427.
6. Shimotohno, K., Takahashi, Y., Shimizu, N., Gojobori, T., Golde, D. W., EL Gessain, A., Lemasson, I., Mesnard, J. M., Barbeau, B. and Mahieux, R. 1985. Proc. Natl. Acad. Sci. USA, 82, 3101.
7. Kwaan, N., Lee, T. H., Chafets, D. M., Nass, C., Newman, B., Smith, J., Garratty, G., Murphy E. L. and HTLV Outcomes Study (HOST) Investigators. 2006, J. Infect. Dis., 194, 1557.
8. Ciminale, V., D'Agostino, D. M., Zotti, L., Franchini, G., Felber, B. K. and Chieco-Bianchi L. 1995, Virol., 209, 445.
9. Rende, F., Cavallari, I., Romanelli, M. G., Diani, E., Bertazzoni, U. and Ciminale, V. 2012, Leuk. Res. Treatment, 2012, 876153.
10. Bender, C., Rende, F., Cotena, A., Righi, P., Ronzi, P., Cavallari, I., Casoli, C., Ciminale, V. and Bertazzoni, U. 2012, Retrovirology, 9, 74.
11. Gaudray, G., Gachon, F., Basbous, J., Biard-Piechazyk, M., Devaux, C. and Mesnard, J. 2002, J. Virol., 76, 12813-22.
12. Cavanagh, M., Landry, S., Audet, B., Arpin-André, C., Hivin, P., Paré, M., Thête, J., Wattel, E., Marriott, S. J., Mesnard, J. and Barbeau, B. 2006, Retrovirology, 3, 15.
13. Manel, N., Kim, F. J., Kinet, S., Taylor, N., Sitbon, M. and Battini, J.-L. 2003, Cell, 114, 449.
14. Jones, K. S., Fugo, K., Petrow-Sadowski, C., Huang, Y., Bertolette, D. C., Lisinski, I., Cushman, S. W., Jacobson, S. and Ruscetti, F. W. 2006, J. Virol., 80, 8291.
15. Igakura, T., Stinchcombe, J. C., Goon, P. K., Taylor, G. P., Weber, J. N., Griffiths, G. M., Tanaka, Y., Osame, M. and Bangham, C. R. 2003, Science, 299, 1713.
16. Pique, C. and Jones, K. S. 2012, Front. Microbiol., 3, 378.
17. Cavallari, I., Rende, F., Bender, C., Romanelli, M. G., D'Agostino, D. M. and Ciminale V. 2013. Front. Microbiol., 4, 235.
18. Catalan-Soares, B., Barbosa-Stancioli, E. F., Alcantara, L. C., Carneiro-Proietti, A. B., Martins, M. L., Namen-Lopes, M. S., Galvão-Castro, B., Ferreira, C. E., Costa, M. C., Pinheiro, S. R., Proietti, F. A. and GIPH (Interdisciplinary HTLV-I/II Research Group). 2005, AIDS Res. Hum. Retroviruses, 21, 521.
19. Shimotohno, K., Takano, M., Terunchi, T. and Miwa, M. 1986, Proc. Natl. Acad. Sci., 83, 8112.
20. Sheehy, N., Lillis, L., Watters, K., Lewis, M., Gautier, V. and Hall W. 2006, Retrovirology, 3, 20.
21. Feuer, G. and Green, P. L. 2005, Oncogene, 24, 5996.
22. Ross, T. M., Minella, A. C., Fang, Z. Y., Pettiford, S. M. and Green, P. L. 1997, J. Virol., 71, 8912.
23. Bertazzoni, U., Turci, M., Avesani, F., di Gianfranco, G., Bidoia, C. and Romanelli, M. G. 2011, Viruses, 3, 541.
24. Higuchi, M. and Fujii, M. 2009, Retrovirology, 6, 117.
25. Basbous, J., Bazarbachi, A., Granier, C., Devaux, C. and Mesnard, J. M. 2003, J. Virol., 77, 13028.
26. Jin, D. Y. and Jeang, K. T. 1997, Nucleic Acids Res., 25, 379.
27. Boxus, M., Twizere, J. C., Legros, S., Dewulf, J. F., Kettmann, R. and Willems, L. 2008, Retrovirology, 5, 76.
28. Shoji, T., Higuchi, M., Kondo, R., Takahashi, M., Oie, M., Tanaka, Y., Aoyagi, Y. and Fujii, M. 2009, Retrovirology, 6, 83.

29. Higuchi, M., Tsubata, C., Kondo, R., Yoshida, S., Takahashi, M., Oie, M., Tanaka, Y., Mahieux, R., Matsuoka, M. and Fujii, M. 2007, J. Virol., 81, 11900.
30. Xiao, G., Cvijic, M. E., Fong, A., Harhaj, E. W., Uhlik, M. T., Waterfield, M. and Sun, S. C. 2001, EMBO J., 20, 6805.
31. Mahieux, R., Pise-Masison, C. A., Lambert, P. F., Nicot, C., De Marchis, L., Gessain, A., Green, P., Hall, W. and Brady, J. N. 2000. J. Virol., 74, 6866.
32. Semmes, O. J., Majone, F., Cantemir, C., Turchetto, L., Hjelle, B. and Jeang, K. T. 1996, Virol., 217, 373.
33. Tripp, A., Liu, Y., Sieburg, M., Montalbano, J., Wrzesinski, S. and Feuer, G. 2003, J. Virol., 77, 12152
34. Tripp, A., Banerjee, P., Sieburg, M., Planelles, V., Li, F. and Feuer, G. 2005, J Virol., 79, 14069.
35. Ciminale, V., Zotti, L., D'Agostino, D. M. and Chieco-Bianchi, L. 1997, J. Virol., 71, 2810.
36. Narayan, M., Younis, I., D'Agostino, D. M. and Green, P. L. 2003, J. Virol., 77, 12829.
37. Nosaka, T., Siomi, H., Adachi, Y., Ishibashi, M., Kubota, S., Maki, M. and Hatanaka, M. 1989, Proc. Natl. Acad. Sci. USA, 86, 9798.
38. Nosaka, T., Miyazaki, Y., Takamatsu, T., Sano, K., Nakai, M., Fujita, S., Martin, T. E. and Hatanaka, M. 1995, Exp. Cell. Res., 219, 122.
39. Nakano, K. and Watanabe, T. 2012, Front. Microbiol., 3, 330.
40. Younis, I. and Green P. L. 2005, Front. Biosci., 10, 431.
41. Yamamoto, B., Li, M., Kesic, M., Younis, I., Lairmore, M. D. and Green P. L. 2008, Retrovirology, 5, 38.
42. Johnson, J. M., Mulloy, J. C., Ciminale, V., Fullen, J., Nicot, C. and Franchini, G. 2000, AIDS Res. Human. Retroviruses, 16, 1777.
43. Younis, I., Khair, L., Dundr, M., Lairmore, M. D., Franchini, G. and Green, P. L. 2004, J. Virol., 78, 11077.
44. Yin, H., Kannian, P., Dissinger, N., Haines, R., Niewiesk, S. and Green, P. L. 2012, J. Virol., 86.8412.
45. Marban, C., McCabe, A., Bukong, T. N., Hall, W. W. and Sheehy, N. 2012, Retrovirology, 9, 98.
46. Sullivan, M. T., Williams, A. E., Fang, C. T., Grandinetti, T., Poiesz, B. J. and Ehrlich, G. D. 1991, Arch. Intern. Med., 151, 2043.
47. Roucoux, D. F. and Murphy, E. L. 2004, AIDS. Rev., 6, 144.
48. Proietti, F. A., Carneiro-Proietti, A. B., Catalan-Soares, B. C. and Murphy, E. L. 2005, Oncogene, 24, 6058.
49. Schreiber, G. B., Busch, M. P., Kleinman, S. H. and Korelitz, J. J. 1996, N. Engl. J. Med., 334, 1685.
50. Glynn, S. A., Kleinman, S. H., Schreiber, G. B., Busch, M. P., Wright, D. J., Smith, J. W., Nass, C. C., Williams, A. E. and Retrovirus Epidemiology Donor Study (REDS). 2000, JAMA, 284, 229.
51. Beilke, M. A. 1992, J. Virol. Methods, 40, 133.
52. Fujiyoshi, T., Li, H. C., Lou, H., Yashiki, S., Karino, S., Zaninovic, V., Oneegllo, S. G., Camacho, M., Andrade, R., Hurtado, L. V., Gomez, L. H., Damiani, E., Cartier, L., Dipierri, J. E., Hayami, M., Sonoda, S. and Tajima, K. 1999. AIDS. Res. Hum. Retroviruses, 15, 1235.
53. Gessain, A. and de Thé, G. J. 1996, Acquir. Immune. Defic. Syndr. Hum. Retrovirol., 13, S228.
54. Heneine, W., Kaplan, J. E., Gracia, F., Lal, R., Roberts, B., Levine, P. H. and Reeves, W. C. 1991, N. Engl. J. Med., 324, 565.
55. Biggar, R. J., Taylor, M. E., Neel, J. V., Hjelle, B., Levine, P. H., Black, F. L., Shaw, G. M., Sharp, P. M. and Hahn, B. H. 1996, Virology, 216, 165.
56. Vandamme, A. M., Bertazzoni, U. and Salemi, M. 2000, Gene, 261, 171.
57. Feigal, E., Murphy, E., Vranizan, K., Bacchetti, P., Chaisson, R., Drummond, J. E., Blattner, W., McGrath, M., Greenspan, J. and Moss, A. 1991, J. Infect. Dis., 164, 36.
58. Salemi, M., Vandamme, A. M., Gradozzi, C., Van Laethem, K., Cattaneo, E., Taylor, G., Casoli C., Goubau, P., Desmyter, J. and Bertazzoni, U. 1998. J. Mol. Evol., 46, 602.

59. Zunt, J. R., Tapia, K., Thiede, H., Lee, R. and Hagan, H. 2006. Scand. J. Infect. Dis., 38, 654.
60. Kalyanaraman, V. S., Sarngadharan, M. G., Robert-Guroff, M., Miyoshi, I., Golde, D. and Gallo, R. C. 1982, Science, 218, 571.
61. Dooneief, G., Marlink, R., Bell, K., Marder, K., Renjifo, B., Stern, Y. and Mayeux, R. 1996, Neurology, 46, 1556.
62. Biswas, H. H., Engstrom, J. W., Kaidarova, Z., Garratty, G., Gibble, J. W., Newman, B. H., Smith, J. W., Ziman, A., Fridey, L., Sacher, R. A. and Murphy, E. L. 2009, Neurology, 73, 781.
63. Bartman, M. T., Kaidarova, Z., Hirschkorn, D., Sacher, R. A., Fridey, J., Garratty, G., Gibble, J., Smith, J. W., Newman, B., Yeo, A. E. and Murphy, E. L., HTLV Outcomes Study (HOST) Investigators. 2008, Blood, 112, 3995.
64. Beilke, M. A. and Barrios C. S. 2007, ACP Medicine, D. C. Dale (Ed.), WebMD Professional Publishing, New York.
65. Murphy, E. L. and Biswas, H. H. 2010, Principles and Practice of Infectious Diseases, G. Mandell, J. Bennett and J. R. Dolin (Eds.), Churchill Livingston, Elsevier, Philadelphia.
66. Khabbaz, R. F., Onorato, I. M., Cannon, R. O., Hartley, T. M., Roberts, B., Hosein, B. and Kaplan, J. E. 1992, N Engl. J. Med., 326, 375.
67. Egan J. F., O'Leary B., Lewis M. J., Mulcahy F., Sheehy N., Hasegawa H., Fitzpatrick F., O'Connor J. J., O'Riordan J. and Hall W. W. 1999, AIDS Res. Hum. Retroviruses, 15, 699.
68. Hershow, R. C., Galai, N., Fukuda, K., Graber, J., Vlahov, D., Rezza, G., Klein, R. S., Des Jarlais, D. C., Vitek, C., Khabbaz, R., Freels, S., Zuckerman, R., Pezzotti, P. and Kaplan, J. E. 1996, J. Infect. Dis., 174, 309.
69. Salemi, M., Cattaneo, E., Casoli, C. and Bertazzoni, U. 1995. J. AIDS. Hum. Retrovirol., 8, 516.
70. Brites, C., Alencar, R., Gusmão, R., Pedroso, C., Netto, E. M., Pedral-Sampaio, D. and Badaró, R. 2001, AIDS, 15, 2053.
71. Figueroa, J. P, Ward, E., Morris, J., Brathwaite, A. R., Peruga, A., Blattner, W., Vermund, S. H. and Hayes, R. 1997, J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol., 15, 232.
72. Hishida, O., Ayisi, N. K., Aidoo, M., Brandful, J., Ampofo, W., Osei-Kwasi, M., Ido, E., Igarashi, T., Takehisa, J. and Miura, T. 1994, AIDS, 8, 1257.
73. Beilke, M. A., Japa, S., Moeller-Hadi, C. and Martin-Schild, S. 2005, Clin. Inf. Dis., 41, e57.
74. Brites, C., Sampalo, J. and Oliveira, A. 2009, AIDS Rev., 11, 8.
75. Briggs, N. C., Battjes, R. J., Cantor, K. P., Blattner, W. A., Yellin, F. M., Wilson, S., Ritz, A. L., Weiss, S. H. and Goedert, J. J. 1995, J. Infect. Dis., 172, 51.
76. Beilke, M. A, Theall, K. P., O'Brien, M., Clayton, J. L., Benjamin, S. M., Winsor, E. L. and Kissinger, P. J. 2004, Clin. Infect. Dis., 39, 256.
77. Turci, M., Pilotti, E., Ronzi, P., Magnani, G., Boschini, A., Parisi, S. G., Zipeto, D., Lisa, A., Casoli, C. and Bertazzoni, U. J. 2006, Acquir. Immune. Defic. Syndr., 41, 100.
78. Bassani, S., López, M., Toro, C., Jiménez, V., Sempere, J. M., Soriano, V. and Benito, J. M. 2007, Clin. Infect. Dis., 44, 105.
79. Zagury, D., Lachgar, A., Chams, V., Fall, L. S., Bernard, J., Zagury, J. F., Bizzini, B., Gringeri, A., Santagostino, E., Rappaport, J., Feldman, J. M., O'Brien, S. J., Burny, A. and Gallo, R. C. M. 1998, Proc. Natl. Acad. Sci. USA, 95, 3857.
80. Cocchi, F., DeVico A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C. and Lusso, P. 1995, Science, 270, 1811.
81. Lehner, T., Wang, Y., Cranage, M., Bergmeier, L. A., Mitchell, E., Tao, L., Hall, G., Dennis, M., Cook, N., Brookes R., Klavinskis, L., Jones, I., Doyle, C. and Ward, R. 1996, Nat. Med., 2, 767.
82. Lehner, T., Wang, Y., Cranage, M., Tao, L., Mitchell, E., Bravery, C., Doyle, C., Pratt, K., Hall, G., Dennis, M., Villinger, L. and Bergmeier, L. 2000, Immunology, 99, 569.

83. Pilotti, E., Elviri, L., Vicenzi, E., Bertazzoni, U., Re, M. C., Allibardi, S., Poli, G. and Casoli, C. 2007, Blood, 109, 1850.
84. Casoli, C., Pilotti, E. and Bertazzoni, U. 2007, AIDS Rev., 9, 140.
85. Barrios, C. S., Abuerreish, M., Castillo, L., Lairmore, M. D., Giam, C. Z. and Beilke, M. A. 2011, Viral. Immunol., 24, 429.
86. Balistrieri, G., Barrios, C. S., Castillo, L., Umunakwe, T., Giam, C. Z. and Beilke, M. A. 2013, Viral. Immunol., 26, 3.
87. Barrios, C. S., Castillo, L., Giam, C. Z., Wu, L. and Beilke, M. A. 2013, AIDS Res. Hum. Retroviruses, 29, 1061.
88. Zhang, W., Chaloner, K., Tillmann, H. L., Williams, C. F. and Stapleton, J. T. 2006, HIV Med., 7, 173-80,
89. Xiang, J., George, S. L., Wunschmann, S., Chang, Q., Klinzman, D. and Stapleton J. T. 2004, Lancet, 363, 2040.
90. Xiang, J., Sathar, M. A., McLinden, J. H., Klinzman, D., Chang Q. and Stapleton J. T. 2005, J. Infect. Dis., 192, 1267.
91. Moss, W. J., Ryon, J. J., Monze, M., Cutts F., Quinn, T. C. and Griffin, D. E. 2002, J. Infect. Dis., 185, 1035.
92. García, M., Yu, X. F., Griffin, D. E. and Moss, W. J. 2005, J. Virol., 79, 9197.
93. Takeuchi O. and Akira, S. 2009, Immunol. Rev., 227, 75.
94. Ijichi, S., Ramundo, M. B., Takahashi, H. and Hall, W. W. J. 1992. Exp. Med., 176, 293.
95. Lal, R. B., Owen, S. M., Rudolph, D. L., Dawson, C. and Prince, H. 1995, Virology, 210, 441.
96. Oliveira, A. L. A., Waters, A., Schor, D., Leite, A. C., Araújo, A. and Hall W. W. 2008, FASEB. J., S22, 503.
97. Norris, P. J., Hirschkorn D. F., Devita D. A., Lee T. H. and Murphy E. L. 2010, Virulence, 1, 19.
98. Ferbas, J., Giorgi, J. V., Amini, S., Grovit-Ferbas, K., Wiley, D. J., Detels, R. and Plaeger, S. 2000, J. Infect. Dis., 182, 1247.
99. Paxton, W. A., Martin, S. R., Tse, D., O'Brien, T. R., Skurnick, J., VanDevanter, N. L., Padian, N., Braun, J. F., Kotler, D. P., Wolinsky, S. M. and Koup, R. A. 1996, Nat. Med., 2, 412.
100. Cocchi, F., DeVico, A. L., Yarchoan, R., Redfield, R., Cleghorn, F., Blattner, W. A., Garzino-Demo, A., Colombini-Hatch, S., Margolis, D. and Gallo, R. C. 2000, Proc. Natl. Acad. Sci. USA, 97, 13812.
101. Garzino-Demo, A., Moss, R. B., Margolick, J. B., Cleghorn, F., Sill, A, Blattner, W. A., Cocchi, F., Carlo, D. J., DeVico, A. L. and Gallo, R. C. 1999, Proc. Natl. Acad. Sci. USA, 96, 11986.
102. Ullum, H., Cozzi Lepri, A., Victor, J., Aladdin, H., Phillips A. N., Gerstoft, J., Skinhøj, P. and Pedersen, B. K. 1998, J. Infect. Dis., 177, 331.
103. Lewis, M. J., Gautier, V. W., Wang, X. P., Kaplan, M. H. and Hall, W. W. 2000, J. Immunol., 165, 4127.
104. Bovolenta, C., Pilotti, E., Mauri, M., Turci, M., Ciancianaini, P., Fisicaro, P., Bertazzoni, U., Poli, G. and Casoli, C. 2002, Blood, 99, 224.
105. Casoli, C., Vicenzi, E., Cimarelli, A., Magnani, G., Ciancianaini, P., Cattaneo, E., Dall'Aglio, P., Poli, G. and Bertazzoni, U. 2000, Blood, 95, 2760.
106. Buckner, C., Roberts, C. R., Foung, S. K., Lipka, J., Reyes, G. R., Hadlock, K., Chan, L., Gongora-Biachi, R. A., Hjelle, B. and Lal, R. B. 1992, J. Infect. Dis., 166, 1160.
107. Chen, J., Petrus, M., Bryant, B. R., Phuc Nguyen, V., Stamer, M., Goldman, C. K., Bamford, R., Morris, J. C., Janik, J. E. and Waldmann, T. A. 2008, Blood, 111, 5163.
108. Waldele, K., Schneider, G., Ruckes, T. and Grassmann, R. 2004, J. Virol., 78, 6081-90.
109. Azimi, N., Brown, K., Bamford R. N., Tagaya, Y., Siebenlist U. and Waldmann, T. A. 1998, Proc. Natl. Acad. Sci. USA, 95, 2452.
110. Mariner, J. M., Lantz, V., Waldmann, T. A. and Azimi, N. 2001. J. Immunol., 166, 2602.
111. Mizuguchi, M., Asao, H., Hara, T., Higuchi, M., Fujii, M. and Nakamura, M. 2009, J. Biol. Chem., 284, 25501.
112. Mori, N., Mukaida, N., Ballard, D. W., Matsushima., K. and Yamamoto., N. 1998, Cancer. Res., 58, 3993.
113. Mori, N., Krensky, A. M., Ohshima, K., Tomita, M., Matsuda, T., Ohta, T., Yamada, Y., Tomonaga, M., Ikeda, S. and Yamamoto, N. 2004, Int. J. Cancer, 111, 548.

114. Hieshima, K., Nagakubo, D., Nakayama, T., Shirakawa, A. K., Jin, Z. and Yoshie, O. 2008, J. Immunol., 180, 931.
115. Nagakubo, D., Jin, Z., Hieshima, K., Nakayama, T., Shirakawa, A. K., Tanaka, Y., Hasegawa, H., Hayashi, T., Tsukasaki, K., Yamada, Y. and Yoshie, O. 2007, Int. J. Cancer, 120, 1591.
116. Jin, Z., Nagakubo, D., Shirakawa, A. K., Nakayama, T., Shigeta, A., Hieshima, K., Yamada, Y. and Yoshie, O. 2009, Int. J. Cancer, 125, 2229.
117. Harhaj, E. W., Harhaj, N. S., Grant, C., Mostoller, K., Alefantis, T., Sun, S. C. and Wigdahl, B. 2005, Virology, 333, 145.
118. Maruyama, M., Shibuya, H., Harada, H., Hatakeyama, M., Seiki, M., Fujita, T., Inoue, J., Yoshida, M. and Taniguchi, T. 1987, Cell, 48, 343.
119. Yao, J. and Wigdahl, B. 2000, Front. Biosci., 5, D138.
120. Bertazzoni, U., Turci, M., Avesani, F., Di Gennaro, G., Bidoia, C. and Romanelli, M. G. 2011, Viruses, 3, 541.
121. Journo, C., Filipe, J., About, F., Chevalier, S. A., Afonso, P. V., Brady, J. N., Flynn, D., Tangy, F., Israël, A., Vidalain, P. O., Mahieux, R. and Weil, R. 2009, PloS. Pathog., 5, e1000521.
122. Avesani, F., Romanelli, M. G., Turci, M., Di Gennaro, G., Sampaio, C., Bidoia, C., Bertazzoni, U. and Bex, F. 2010, Virology, 408, 39.
123. Barrios, C. S., Castillo, L., Huijun, Z., Giam, C. Z. and Beilke, M. A. 2014, Clin. Exp. Immunol., 175, 92.
124. Rollenhagen, C. and Asin, S. N. 2010, J. Acquir. Immune Defic. Syndr., 54, 463.
125. Lambert, J. S., Machado, E. S., Watson, D. C., Sill, A. M., Lim, J. K., Charurat, M., Cunha, S. M., Afonso, A. O., Oliviera, R. H., Tanuri, A. and DeVico, A. L. 2007, Pediatr. Infect. Dis. J., 26, 935.
126. Dinarello, C. A. 2000, Chest, 118, 503.
127. Takashima, H., Eguchi, K., Kawakami, A., Kawabe, Y., Migita, K., Sakai, M., Origuchi, T. and Nagataki, S. 1996, Annu. Rheum. Dis., 55, 632.
128. Banerjee, P., Rochford., R, Antel, J., Canute, G., Wrzesinski, S., Sieburg, M. and Feuer, G. 2007, J. Virol., 81, 1690.
129. La, R. B. and Rudolph, D. L. 1991, Blood, 78, 571.
130. Prendergast, A., Prado, J. G., Kang, Y. H., Chen, F., Riddell, L. A., Luzzi, G., Goulder, P. and Klenerman, P. 2010, AIDS, 24, 491.
131. Singh A., Vajpayee M., Ali S. A., Mojumdar K., Chauhan, N. K. and Singh, R. 2012, Cytokine, 60, 55.
132. Zhao, T., Satou, Y., Sugata, K., Miyazato, P., Green, P. L., Imamura, T. and Matsuoka, M. 2011, Blood, 118, 1865.
133. Malhotra, N. and Kang, J. 2013, Immunology, 139, 1.