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The leukemogenic activity of Tax_{HTLV-1} during human alphabeta T cell development

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TABLE OF CONTENTS

1. ABSTRACT

The regulatory Tax protein of HTLV-1 (Human T-cell Leukaemia Virus type 1) is critically involved in the initiation of ATL (adult T-cell leukaemia). Indeed, Tax provides infected T-cells with a growth advantage and with the potential to get transformed through the deregulation of cell-cycle progression and the acquisition of genetic alterations. Considering that leukemias are induced by disturbances in hematopoietic cells development, we hypothesize that the expression of Tax in human immature thymocytes is a prerequisite to the emergence of ATL cells. Studies of alphabeta T-cell development in the thymus have shown that beta-selection, an early important checkpoint, is regulated by transcription factors that are decisive in the control of cell proliferation, differentiation and survival. Interestingly, Tax is endowed with the ability to interfere with the activity of these transcription factors. We therefore propose that the HTLV-1 infection of these specific target thymocytes leads to a transcriptional deregulation of early alphabeta T cell development, thus inducing a pre-leukemogenic event that favours the subsequent proliferation of ATL cells.

2. INTRODUCTION

Unravelling the cellular and molecular mechanisms of tumour development represents an immense challenge to which numerous investigators in cancer research are daily confronted. During the last thirty years of the 20th century, cancer has emerged as a genetic disease, thanks to the identification of genes, which when mutated, have been shown to be implicated in tumour induction. Clearly, alterations of three types of genes were found responsible for tumorigenesis: oncogenes, tumor-suppressor genes and stability genes (1). Furthermore, these observations have provided overwhelming evidence that virtually all cancers are derived from a monoclonal outgrowth. Indeed, they were found to be the progeny of a single cell and to develop through a serie of successive stages. In that context, studies with oncogenic retroviruses, which were first discovered in association with leukemia, have largely contributed to an understanding of tumour induction. They have indeed paved the way to the discovery of viral oncogenes (v-onc), and have helped to underline that mutation and chromosomal translocation of their cellular homologues (c-onc or proto-oncogene) are

contributing to tumorigenesis. Furthermore, as observed with most retroviruses, they are associated with a specific type of tumour, despite being able to infect a variety of cell types *in vivo* and *in vitro.*

Consequently, one of the fundamental problems in cancer research concerns the identification of the normal cell in which cancer initiates. This target cell specificity gets its interest especially in hematopoietic cell tumours arising at specific stages of hematopoietic cell development. Those kinds of tumours are associated with leukaemia viruses either containing *v-onc* genes or favouring insertional mutagenesis. Deciphering these mechanisms has greatly contributed to clarify the ways in which the retroviruses involved affect proliferation and differentiation of the target cells, thus triggering the leukemogenic process.

In humans, the first and unique retrovirus identified by its association with a neoplastic disease is HTLV-1 (Human T cell Leukaemia/lymphoma Virus, type 1), the etiologic agent of Adult T-cell Leukaemia (ATL), a leukaemia of peripheral, mature $CD4^+$ T cells (2). This unique clinical entity has been described in individuals living in clustered areas of southwest Japan, suggesting that a virus might be at the origin of this pathology. A few years later, this hypothesis was confirmed by the isolation of retroviral particles from a culture of T lymphocytes derived from a patient diagnosed with cutaneous T-cell lymphoma. HTLV-1, that belongs to the deltaretrovirus family, is a replication-competent retrovirus. Its provirus, flanked by the two LTR (Long Terminal Repeat) sequences, contains genes coding for structural and enzymatic proteins (Gag, Env, Reverse Transcriptase, Protease and Integrase). In addition, specific sequences lying between the *env* gene and the 3'LTR, first referred to as the pX region, were then shown to encompass, through alternative splicing of the genomic RNA, a series of open reading frames (ORFs I-IV), that encode several non-structural proteins (Tax, Rex, p12, p13, p30 and p21). These proteins are either regulating the viral life cycle, at the transcriptional and/or post-transcriptional levels, or exerting functional effects on the infected cells (3, 4). Recently, a novel ORF identified in the minus strand of the pX region has been shown to encode a protein named HBZ (for HTLV-1 bZIP), containing a leucine zipper motif (bZip) in its C-Terminal region, that allows interactions with other bZIP cellular proteins (5-7). Among the non-structural proteins, the 40 kDa Tax nuclear phosphoprotein has been shown to be essential for viral replication as well as in the expression of many phenotypic features of HTLV-1-transformed T cells, playing a prominent role in the lymphoproliferative events triggered by HTLV-1 infection.

After proceeding to a survey of the main concepts of leukemic development, the present review will deal with the effects of Tax, the best studied of HTLV-1 regulatory proteins, on T-cell immortalization and transformation, and with its specific role during the development of ATL. We will present data from immunological, cellular and molecular studies that have helped to determine when and how the pleiotropic effects of the regulatory Tax protein are involved in the development of ATL. In particular, we will evaluate whether the activity of Tax in specific target cells such as T-cell precursors in the human thymus is linked to the initiation and to the promotion of the leukemogenic process.

3. TUMORIGENESIS AND THE LEUKEMOGENIC PROCESS

As stated above, tumorigenesis is often described as a multistep process (8). According to experimental studies that apply carcinogens to animals, it has been distinguished the stage of initiation as starting tumour development**,** that of promotion as stimulating the following steps and that of final progression as invasion through metastasis. Commonly, initiation implies that cells acquire not only a growth advantage, but also the potential to get transformed. Genetic modifications are considered to play a major role at this stage. They generally involve constitutive activation of a proto-oncogene, by genetic translocation or integration of a retrovirus genome upstream of a proto-oncogene, leading to the disruption of the host genome integrity and unregulated expression of the considered gene. Alternatively, viruses encoding regulatory proteins can activate proliferation and/or inhibit tumour suppressor genes and/or stability genes such as genes of the DNA reparation machinery.

During the promotion stage, the growth advantage given by the initial genetic alterations will favour the emergence of a monoclonal outgrowth of cells, which are able to endure additional stimuli, leading to the perturbation of mechanisms protecting from uncontrolled proliferation (1, 9). In that context, numerous studies have proposed that the signalling pathways disrupted in tumour cells converge to a deregulation of transcription factors that will ultimately be responsible for an altered expression of numerous cellular genes (10). Furthermore, among the alterations and mutations that contribute to the progression of the disease, a specific attention has been made on telomerase activity for its role in the maintenance of telomere length. Indeed, shortening of telomeres during a normal lifespan is responsible for the emergence of genetic instability that could participate to the oncogenic process. Generally, cells go to senescence while reaching a critical length of their telomeres. However, the reactivation of telomerase activity is a common feature of cancer cells and contributes not only in the maintenance of short telomeres, but also in the proliferation of tumour cells (11). Finally, a last event is required for the conversion into an invasive tumour that escapes from the host immune response, conversion that might result from additional mutations of genes involved in invasion, angiogenesis and metastasis (9).

Leukaemia/lymphoma are defined as the proliferation of a malignant clone in the central haematopoietic system or in peripheral lymphopoietic organs, respectively. Composed of mobile neoplastic cells, they have a greater invading potential by comparison with carcinomas and sarcomas, which originate from epithelial

or mesenchymal attached cells. Most of the understanding of leukemogenesis in humans has been extrapolated from the analysis of human leukaemic cell lines and of mouse models. These observations have led to the identification and characterization of the genes involved in triggering leukaemia/lymphoma and have provided evidence for the occurrence of multiple cooperating events (12). As for other types of tumours, leukaemia/lymphoma are very often characterized by an aberrant expression of transcription factors, explaining the altered expression of numerous cellular genes controlling the maturation of haematopoietic cells (10). Such a dysregulation commonly leads to a disrupted differentiation of the corresponding haematopoietic lineage as one critical event of the leukemogenic process. Accordingly, the emergence of the malignant monoclonal population might be specific of the target cells in which this dysregulation is occurring (12).

4. ATL, A LEUKEMOGENIC PROCESS ASSOCIATED WITH HTLV-1 INFECTION

Commonly, only 2 to 5% of people infected with HTLV-1 will develop ATL. Epidemiological surveys have indeed indicated that most of the 15-20 millions of infected individuals remain seropositive "healthy" carriers during their entire life without any pathological sign. Such a state has been linked to the development of humoral and cytotoxic responses that lower expression of viral antigens, and specially that of Tax (13, 14).

ATL has unique clinical, pathological and cytological features. ATL patients are classified into four clinical subtypes: smoldering, chronic, acute and lymphoma type (15). Little or mild clinical symptoms characterize smoldering patients, while those with chronic or acute leukaemia/lymphoma show a rapid progression and common association with lymphoadenopathy, hepatosplenomegaly and hypercalcemia, frequent skin lesions and resistance to treatment with current anti-leukemia agents (2). The number of leukemic cells that increases according to the severity of the disease display peculiar polymorphic nuclei. ATL cells belong to the T-cell lineage with CD4 surface expression, a helper-inducer marker, and other mature T-cell markers such as CD2 and CD3, CD3/ T-cell receptor (TCR) molecules on cell surfaces being frequently down-regulated. ATL cells are activated T cells as ascertained by the expression of CD25, the α chain of the interkeukin-2 (IL-2) receptor (IL-2R). The HTLV-1 provirus does not code for any oncogene and proviral integration occurs randomly in the host genome. The HTLV-1 provirus is found monoclonally integrated in ATL cells, confirming that these leukemic cells occur from a single clone of HTLV-1-carrying cells. Furthermore, these cells were not producing viral particles (2). Indeed it was observed that in 90% of ATL cases, the Tax protein is not or poorly expressed because of genetic changes or DNA methylation or deletion of the 5'LTR which functions as the provirus promoter/enhancer (16). Likewise, the telomerase activity has been found to be higher in acute ATL patients than that in healthy blood donors, in HTLV-1 carriers and in chronic ATL patients. Thus, the reactivation of telomerase in peripheral blood mononuclear cells of ATL patients is providing a worsening marker of ATL, especially during the evolution from the chronic to the acute type (17). Finally, the presence of significant shorter telomeres in chronic and acute ATL patients compared to those of the two other subgroups, is pleading for a major telomeric dysfunction that might favour genetic instability.

The risk to develop ATL after HTLV-1 infection appears to be related to age, route of infection and the immune competency of the host. It has been reported that the oral route, i.e. breast-feeding, is one of the main route of viral transmission in humans, and this might be the critical event in the initiation of the long leukemic process (18). Thus, after either oral or intraperitoneal inoculation of immunocompetent adult rats with mitomycin-treated HTLV-1 infected T-cells, HTLV-1-specific antibody responses appear significantly lower in orally infected rats than in intra-peritoneally infected animals, indicating that the route of primary T-cell infection affects host T-cell immunity against HTLV-1. Moreover, lower immunological competence was correlated with higher HTLV-1 proviral load (19, 20). Since oral infection is a major route of vertical HTLV-1 infection, it is inferred that a low immune responsiveness to HTLV-1 might favour the accumulation of the HTLV-1-infected cells. Indeed, infants born to HTLV-1-carrying mothers remain seronegative for HTLV-1 for about 18 months, period characterized by a state of immunological tolerance. Later on, HTLV-1 specific responses that recover in most of these carriers protect from ATL development. However, some vertically infected-HTLV-1 carriers, with a low T-cell response combined to a high viral load, might have high risks to develop ATL. These observations clearly underline that HTLV-1 infection early in life appears as a critical event in the onset of the leukemogenic process.

5. TAX, A REGULATORY PROTEIN WITH PLEIOTROPIC EFFECTS

Initial experimental studies devoted to the biological effects of HTLV-1 have described the progressive changes affecting human primary T cells infected *in vitro*, after coculture with X-irradiated HTLV-1 producing T-cells. During the initial phase (phase I) after infection, cells express the IL-2Rα (CD25), and proliferate in an IL2-dependent manner. They are not morphologically different from the uninfected activated T cells and continue to express the CD3/TCR complex. During this immortalization phase, the infected cells are displaying polyclonal proviral integration. In contrast, the phase II cells, that emerge about three months after infection, have acquired the ability to proliferate in the absence of IL-2, grow in large dense aggregates, have a giant-like appearance and do not express the CD3/TCR complex. Furthermore, the outgrowth of this cell population is characterized by an oligoclonal proviral integration and by a dramatic increase in pX mRNA expression (21 , 22). These HTLV-1-infected T cells also display a high mutation rate and genetic changes, indicating that they have entered the transformation stage. At the difference with ATL cells, most of the *in vitro* HTLV-1-transformed cells are producing viral particles. These observations clearly

underline that HTLV-1 is endowed with the capacity to immortalize and to transform human T cells.

Studies to evaluate the contribution of Tax in the immortalization and transformation events have been undertaken (4, 23). Indeed, the inherent role of Tax is to enhance proviral transcription from the 5' LTR, via the direct interaction with ATF/CREB factors together with the two related transcriptional co-activators, CBP (CREB binding protein)/p300 and p300/CBP-associated factor (PCAF). In addition to its effect on the CREB/ATF factors, Tax initiates and maintains the constitutive activation of the NF-κB pathway and stimulates the transcriptional activity of SRF and AP-1. Interestingly, these observations have unravelled the trans-activating ability of Tax, which is achieved through protein-protein binding and have been linked to post-transcriptional modifications, such as phophorylation, acetylation or sumoylation. They also have led to the discovery of the effects of Tax on the expression of numerous cellular genes essential to the growth and the survival of T cells. Conversely, Tax inactivates gene expression, mainly by negatively interfering with the transcriptional activity of bHLH proteins through its interaction with CBP/p300 co-activators. On the whole, Tax is potentially able, to trigger the proliferation and the survival of cells displaying phenotypic alterations as well as genetic or epigenetic changes that are detrimental when occurring in normal cells. Consequently, Tax might be implicated not only in the initiation of the leukemogenic process, but also in its progression.

However, most of these studies have been performed with lymphoid T as well as with non-lymphoid cell lines (either transiently or stably transfected with vectors expressing Tax or constitutively expressing that viral protein), in which the Tax-induced cellular alterations might depend on pre-existing somatic mutations. Thus, when Tax is expressed in the mouse (CTLL2) and human (Kit225) IL-2 dependent T cell lines, both derived from T-cell lymphomas, these cells acquire the ability to proliferate in the absence of IL-2 (24, 25). Conversely, studies evaluating the effects of Tax in human primary T cells have mainly shown that the stable expression of Tax leads only to their IL-2 dependent proliferation. However, even if these cells do not progress to the IL-2 independent transformation phase, they display some features similar to those of HTLV-1 transformed T cells (26-30). Clearly, these effects may be related to the ability of Tax to activate transcription factors, such as NF-κB or CREB or AP-1, implicated in the expression of genes controlling and/or enhancing cell cycle progression. Collectively, these experiments, although being mainly performed under culture conditions that bypass any selective pressure, converge and confirm that Tax appears as a protein mainly endowed with the ability to promote lymphoproliferative events favouring the accumulation of mutations participating to the emergence of leukemic T-cells that retain phenotypic features (such as NF-kB, AP-1, IL2-R,….) induced by Tax, even after the silencing of this viral protein. However, these observations also propose that the implication of Tax in ATL development is restricted to the early steps following HTLV-1 infection of neonates.

6. THE EARLY ALPHABETA T-CELL DEVELOPMENT: A PRE-TCR-DEPENDENT PROCESS

Hematopoiesis controls the development of the different blood cell lineages through specification and commitment of hematopoietic stem cells. This tightly regulated process is under the control of cell-intrinsic mechanisms such as transcription factors and extrinsic influences such as cytokines and the microenvironment, all acting within a timing-sensitive confine. During the last decade, studies on T-cell development have benefited from investigations on other blood lineages. They have particularly underlined the pivotal influence of transcription factors required for proliferation and differentiation.

Normal αβT-cell development that occurs in the thymus involves progression of early thymic precursors through distinct developmental steps that are defined by the sequential loss or gain of specific cell markers (31). Thus, each specific stage of differentiating thymocytes can be discriminated on the basis of cell-surface antigen expression and on the status of the TCR molecular rearrangements. In humans, specification of multipotent $CD34^+$ T cell precursors that migrate from the bone marrow into the thymus generates T-cell lineage-committed thymocytes that are double-negative (DN) for CD4 and CD8. These DN thymocytes enter a maturation process during which they will proliferate, eventually survive and differentiate into $CD4^+CD8^+$ double-positive (DP) cells that will mature either into single positive (SP) $CD4^+$ or $CD8^+$ T cells that leave the thymus for the periphery and migrate to secondary lymphoid organs (Figure 1).

An early critical checkpoint controls the proliferation and selection of the only DN thymocytes that carry productively rearranged TCRβ locus for further differentiation into DP cells. Such a process is subsequent to the expression of recombinase Rag1/2 proteins involved in the rearrangement of the TCR locus and to the expression of a pre-TCRα (pTα) invariant chain that, in association with the rearranged TCRβ chain and with signal-transducing CD3 molecules forms the pre-TCR complex at the membrane of human immature thymocytes. This pre-TCR dependent checkpoint (also referred to as the β-selection checkpoint) has been shown to begin in a small subset of immature thymocytes, expressing CD4 and known as CD4 ISP (immature single positive) cells. Pre-TCR signalling triggers the MAPK (ras-MAP kinase), PKC (protein kinase C) and PI3K/Akt pathways that activate transcription factors belonging to the rel/NF-κB and AP-1 families involved in the survival, proliferation and differentiation of β-selected DN thymocytes toward the DP stage. After β-selection, the expression of the pTα invariant chain of the pre-TCR declines, thus leading to an arrest of proliferation, concomitant with the differentiation process. Rearrangements of the TCRα locus are initiated, and most of the cells then become functional TCRαβ cells. They are either rescued through the positive selection checkpoint, by a low-affinity interaction of the mature TCR with self-

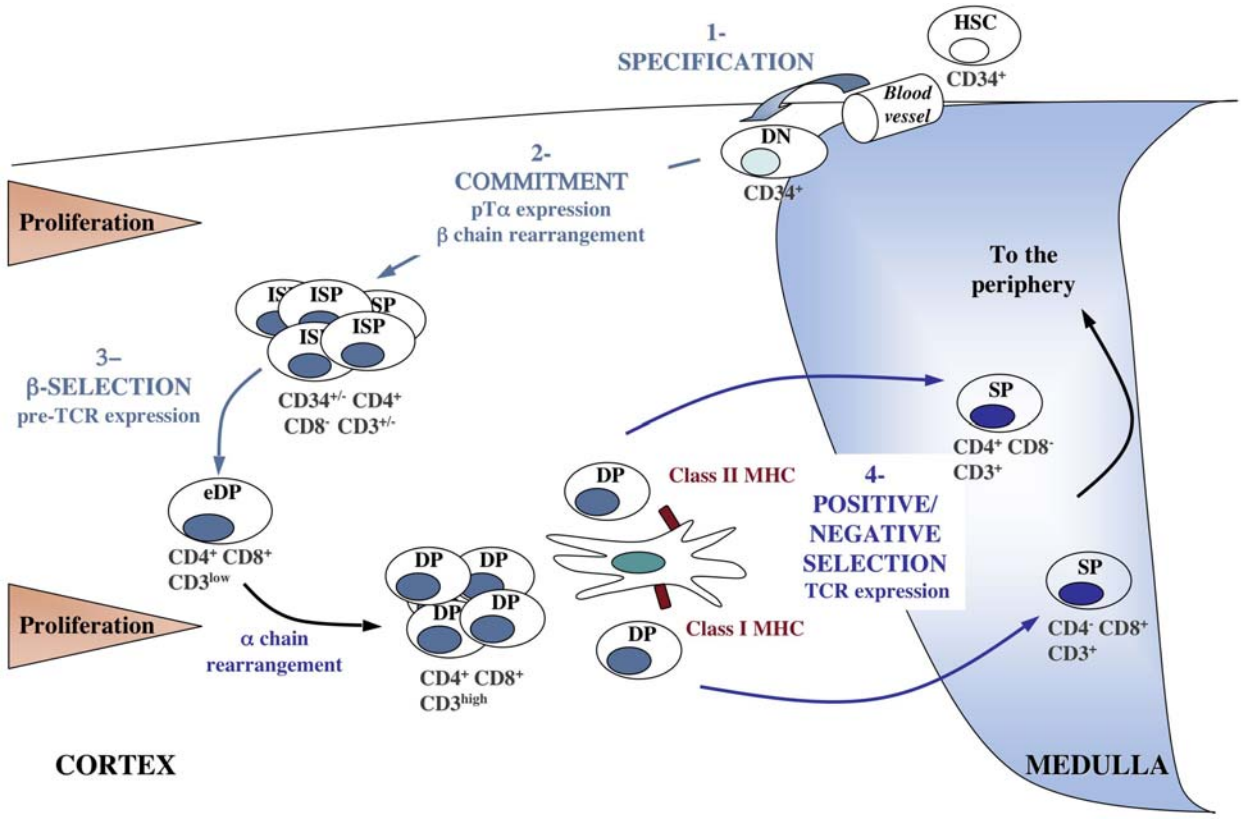

Figure 1. The development of αβ T cells in the human thymus. Normal αβ T-cell development involves progression of early thymic progenitors through distinct developmental steps defined by the sequential loss or gain expression of specific markers. Specification process occurs in $CD34^+$ precursors that enter the thymus. Then, the expression of CD4 concomitant with the rearrangement of the TCRβ locus together with that of the invariant chain pTα and of the β chain in association with CD3 molecules, allows the formation of the pre-TCR (T-cell Receptor) complex during the transition from the CD4 Immature Single Positive ($CD4^{ISP}$) cells to the early DP cells. This β-selection process is followed by an important proliferation wave. After rearrangement of the α locus, the mature TCR is selected through the interaction with MHC molecules at the surface of thymic epithelial cells. Low affinity receptors are positively selected while high affinity receptors are eliminated through negative selection. Then, cells differentiate either in $CD8^+$ or $CD4^+$ Single Positive (SP) cells that leave the thymus to colonize the secondary lymphoid organs.

peptides presented by MHC molecules, or deleted through the negative selection checkpoint if they express high-affinity receptors for self-peptide-MHC.

Thus, proliferation and differentiation of αβ T lymphocytes are mostly dependent on the events presiding the assembly of the pre-TCR and of those triggered by the β-selection process. Indeed, mutant mice that are lacking the Rag1 or Rag2 proteins, TCRβ or pTα cannot form a pre-TCR complex and as a consequence are displaying a developmental arrest at the DN stage. Genetic ablation and overexpression studies in mice have identified a number of molecules that regulate the transition of DN thymocytes to the DP stage. Among them, transcription factors either activated by the Notch and Wnt signalling pathways and or belonging to the basic helix-loop-helix (bHLH) family, are critically involved in the molecular programs that control cell proliferation, differentiation and survival during these early stages of thymocytes development. (32). They are also intimately linked with the induction of leukemogenic events that result in the transformation of T-cell precursors (33).

Both Notch and Wnt signalling pathways are exogenously activated, and act as molecular switches, by allowing the conversion of a transcription factor normally bound to the DNA as a transcriptional repressor into an activator. Notch activation is required for commitment of early thymocytes progenitors to the T-cell lineage as well as during the DN transitional stages. The Notch family of transmembrane receptors encompasses four proteins (Notch1, Notch2, Notch3 and Notch4). The Delta and Jagged family ligand engagement results in the proteolytic release of the intracytoplasmic domain of Notch (Notch-IC). Notch-IC translocates to the nucleus and interacts with the DNA-bound transcription factor CSL (CBF1/Suppressor of Hairless/Lag-1) to regulate a limited number of target genes, among which the *pTα* gene (34).

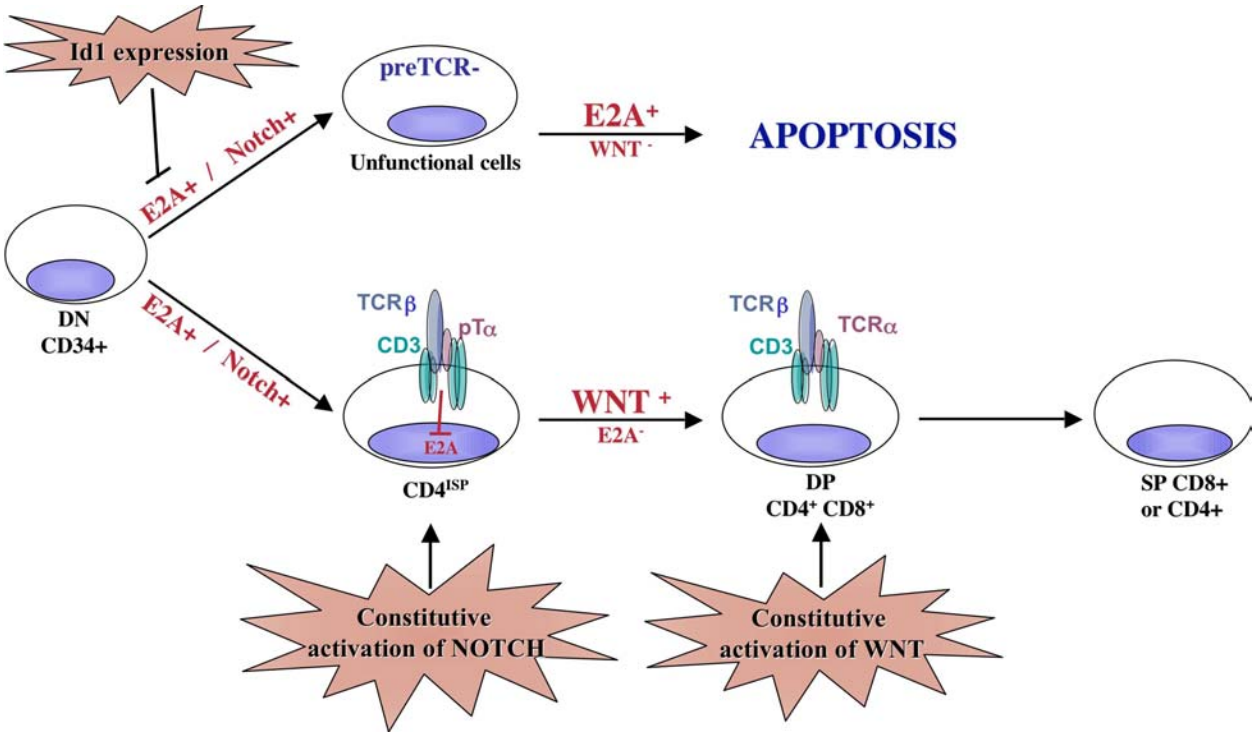

Figure 2. The onset of leukaemogenic events is associated with the disruption of early αβT-cell development. Differentiation of αβ T lymphocytes is mostly dependent on the events triggered by the β-selection process. Such a process involves a balanced expression of specific transcription factors either activated by the Notch and WNT signalling pathways and/or belonging to the bHLH family. As the β-selection checkpoint is ensuring the survival of thymocytes expressing a functional TCR β–chain, it implies that two major signalling pathways act in parallel to promote either apoptosis or survival of these cells. These events are mostly dependent on the expression of the pre-TCR. Notably, the expression of pTα, an essential component of the pre-TCR, relies on the expression of E2A bHLH transcription factors as well as the activation of Notch dependent genes. However, persistent activation of Notch and E2A proteins function as gatekeepers to arrest both proliferation and differentiation of deficient thymocytes. Furthermore, survival and proliferation of pre-TCR expressing cells are partially controlled by the WNT signalling cascade. Numerous studies have shown that the disruption of one of those signalling pathways involves a partial block of differentiation and is associated with the emergence of leukaemia/lymphoma.

The Wnt signalling cascade is required for the development of early thymic progenitors, and has also been implicated in the progression until the DP stage. The Wnt signalling pathway is initiated by the binding of a WNT protein to the cysteine-rich domain of the frizzled (FZ) family and a co-receptor of the low-density-lipoprotein-receptor-related-protein family. The events triggered by the well-defined canonical signalling WNT-FZ pathway are the nuclear translocation of β-catenin and its physical binding to members of the TCF (T-cell factor)/LEF (lymphocyte-enhancer-binding factor) family of transcription factors (35).

Among the bHLH transcription factors, originally identified by their ability to bind E box (CANNTG) elements, E2A proteins have been shown to upregulate the expression of RAG1 and RAG2 genes and more importantly that of the pTα gene, thus participating to the assembly of the pre-TCR complex (36-38). However, they are also required for the complete arrest in both differentiation and proliferation of thymocytes that lack the pre-TCR or key signalling molecules. Interestingly, ectopic expression of E2A proteins in human leukemic T cells inhibits cell-cycle progression and promotes apoptosis, underlining that they function as tumour suppressors. Furthermore, to prevent uncontrolled survival or proliferation the pre-TCR should be eliminated immediately after β-selection. Thus pre-TCR signalling induces the expression of Id proteins, which comprise a class of HLH proteins that lack a DNA-binding domain. The formation of E2A-Id heterodimers unable to bind E-boxes is then followed by a down-regulation of the E2A transcriptional activity and maturation of the selected thymocytes into DP cells (39). By this way, pre-TCR signalling also terminates pTα transcription and abolishes the formation of the pre-TCR complex.

7. LEUKEMOGENIC EVENTS ARISE AROUND THE BETA-SELECTION CHECKPOINT

Because of their importance in the regulation of normal early T-cell development, constitutive activation of Notch and Wnt signals and inhibition of E2A proteins have been shown to lead to the development of T-cell leukaemia/lymphoma (Figure 2).

Deregulated activation of Notch has been etiologically linked to T-cell leukemias in humans and in animal models. More than 50% of all examined cases of human T-ALL (T acute lymphoblastic leukemia) bear Notch-activating mutations. Likewise, transgenic mice expressing constitutively active truncated Notch1 or Notch3 proteins develop T-cell lymphomas. Thymocyte transformation is linked to the inhibition of the E2A pathway and to the up-regulation of c-Myc, through the pre-TCR signalling (40). Thus, Notch-3IC has been shown to promote pre-TCR assembly through the expression of the pTα component and to induce the expression of the bHLH Tal-1 protein. Tal1, also called SCL, belongs to another family of inhibitors of the transcriptional activity of E proteins that contain HLH motifs able to mediate dimerization with E proteins. Interestingly, these heterodimers can bind DNA, but are unable to activate transcription. Pre-TCR signalling will then induce Tal-1 phosphorylation leading to the inhibition of E47/HEB transcriptional activity (41) and the overexpression of cyclin D1 (42). Interestingly, NF-κB is constitutively active in the leukemic T cells of these transgenic mice (40).

Furthermore, the transgenic expression of an activated form of β-catenin at the DN stage stalls the transition from the DP to the SP thymocyte stage and triggers the development of aggressive T-cell lymphomas with a phenotype of DP cells. Lymphomagenesis requires additional secondary genetic events, among them the transcriptional up-regulation of c-Myc, but appears to be independent of Notch mutations (43).

Finally, consistent with the ability of E2A proteins to function as tumour suppressors, experimental inactivation of these transcription factors has been shown to result in the spontaneous development of thymic lymphoma. Thus, in mice, disruption of the E2A gene or inhibition of E2A transcriptional activity by Id proteins initially leads to abnormalities in the earliest stages of αβ T-cell development and to varying degrees of reduced thymic cellularity (37, 44-46). Later in life, these E2A-deficient mice become prone to developing highly malignant T-cell lymphoma. These observations suggest that before the onset of T-cell lymphomas, genetic mutations or epigenetic alterations have accumulated in the surviving thymocytes that undergo leukemic growth. Consequently, inactivation of E2A proteins in immature thymocytes is a predisposing event to the initiation of a lymphoproliferative disease.

Collectively, leukemogenic events initiated during early thymopoiesis coincide with the specific effects of each transcriptional pathway at a peculiar stage of T cell development. Thus, malignant transformation by Notch IC is acting in thymocyte development earlier than Wnt and β-catenin that are inducing lymphomagenesis up until the DP stage. Likewise, the implication of E2A proteins in leukemogenesis is reminiscent of the modulation of their activity before and during the β-selection process. Whatsoever, they propose that the permanent expression of the pre-TCR is favouring the maintenance of convergent leukemogenic pathways.

8. DISRUPTION OF ALPHABETA T-CELL DEVELOPMENT BY TAX: A PRE-LEUKEMOGENIC EVENT?

Acute leukemias have been described to arise from cells having undergone mutations interfering with differentiation and stimulating inappropriate proliferation. This underlines the role played by disturbances of the normal developmental framework of hematopoietic cells at the origin of these diseases. The World Health Organization has classified ATL as a mature T-cell neoplasm, because the leukemic cells are generally $CD4^+$ T lymphocytes. However, less common phenotypes include $CD4^-CD8^-$, $CD8^+$ and $CD4^+CD8^+$, suggesting that aberrant maturation represents a prerequisite for the initiation and development of leukemogenic events (47-49). These observations propose that infection and transformation of thymic progenitors might be critical in the pathogenesis of ATL. They nevertheless imply that the leukemic clone, in spite of having an aberrant developmental hierarchy, retains aspects of normal maturation. We have first approached this hypothesis by investigating whether HTLV-1 might interfere with human αβT-cell development.

Several reports have indicated that HTLV-1 infection within the thymus might represent an essential and critical event of viral pathogenesis. Thus, it was found that HTLV-1 is able to productively infect human hematopoietic $CD34^+$ progenitor cells as well as human immature thymocytes (50, 51). It has been also demonstrated that reconstitution of T lymphopoiesis with HTLV-1-infected $CD34^+$ cells in severe combined immunodeficient mice engrafted with human thymus and liver tissues resulted in the perturbation of thymopoiesis and an aberrant display of thymocyte subpopulations (50).

In line with these observations, rabbits inoculated with HTLV-1 infected T cells show a thymic atrophy in the presence of a rapidly increasing thymic proviral load that preceded the development of an acute ATL-like malignant lymphoproliferative disease (52). More precisely, thymic hypocellularity was observed in transgenic mice expressing HTLV-1 pX regulatory proteins (among which Tax) (53). As recent observations have indicated that Tax inhibits cell proliferation and induces rapid senescence in a wide variety of cells (54, 55), it might then be postulated that Tax is responsible for a thymic atrophy. Conversely, transgenic mice, in which Tax is expressed under the control of the Lck proximal promoter, are developing, after a long latency period, lymphoma and leukemia with features characteristic of acute ATL, such as constitutive activation of NF-κB (56). As the Lck proximal promoter restricts transgene expression to developing thymocytes, these data indicate that Tax should also promote the survival and proliferation of these cells.

Altogether, perturbation of thymocyte maturation may be a consequence of HTLV-1 infection within the thymus. It is plausible that Tax expression in immature thymocytes may interfere with critical transitional stages of early thymopoiesis especially around the β–selection checkpoint. Indeed, Tax, by repressing the transcriptional

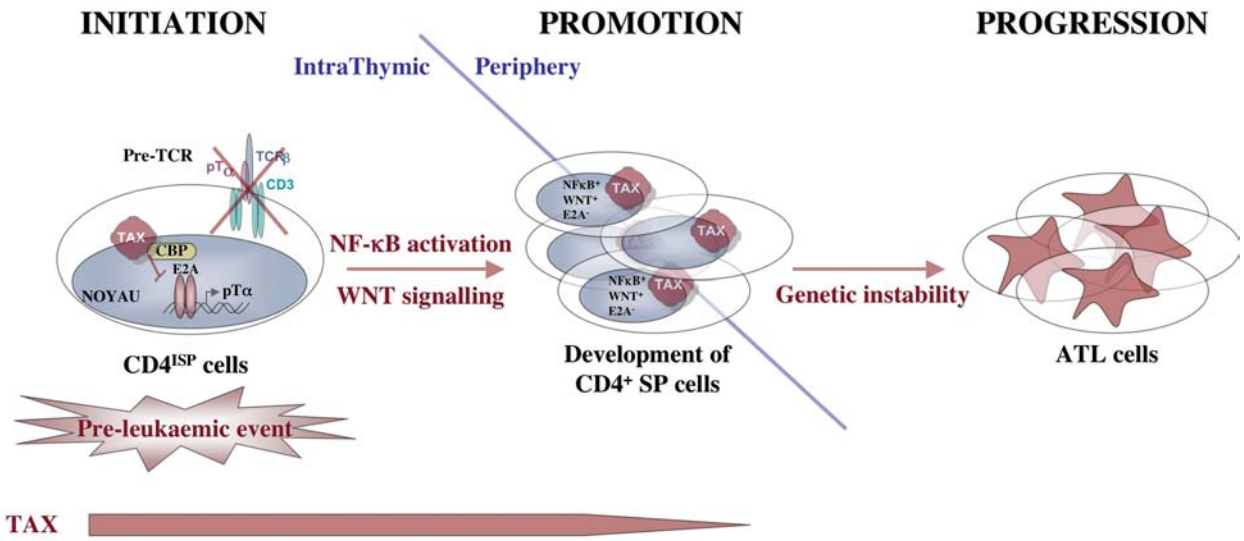

Figure 3. Implication of Tax in the leukemogenic process leading to ATL. A disruption of αβ T cell development might represent the initial event at the onset of ATL. The expression of Tax would induce constitutive activation of signalling pathways, in place of the pre-TCR, thus allowing maturation of infected thymocytes. These cells would be able to sustain additional genetic events leading to the irreversible modification of the cells. Last events, independent of the presence of Tax, would allow the emergence of a malignant clone and its proliferation in the periphery.

activity of E2A proteins in a CBP/p300-dependent manner, is able to down-regulate the expression of the pTα gene in human CD4 ISP thymocytes, thus interfering with the assembly of the pre-TCR complex (57). These observations are the first to show an effect of Tax on a critical event of early T-cell development. As underlined above, the functional inhibition of E2A proteins has been linked to the development of highly malignant T-cell lymphoma (37, 44-46). Interestingly, the expression of GLUT1, one of the identified receptors for HTLVs is restricted to a significant percentage of immature thymocytes undergoing β-selection (58, 59). We thus propose that Tax acts as a silencer of E2A and thus, is implicated in the initiation and promotion of ATL.

Consequently, the main activity of Tax expressed within the thymus might be to disrupt the maturation of developing thymocytes, and to favour the emergence of pre-TCR$^-$ Tax$^+$ cells. The fate of these cells would be therefore dependent on the effects of Tax on survival and/or proliferation. Indeed, they might undergo programmed cell death, as ascertained by thymic hypocellularity. Alternatively, Tax by activating critical signalling pathways, such as NF-κB and Akt, may trigger their proliferation, thus providing a substrate population for secondary mutations and/or further altered gene expression that would allow some clones to grow out malignantly. Preliminary results are in accordance with an activation of NF-κB signalling pathways since the transcription of the anti-apoptotic gene Bfl1 (a known target of NF-kB transcriptional activity (60)) is upregulated in immature thymocytes expressing Tax (Wencker, unpublished data). Furthermore, Tax has been recently found to activate β-catenin through the Akt signalling pathway, pleading that the maturation of thymocytes expressing Tax can occur in the absence of a functional pre-TCR (61). These observations suggest that before the onset of T-cell lymphomas, genetic mutations or epigenetic alterations have accumulated in the surviving thymocytes that can undergo leukemic growth. Collectively, they underline the implication of Tax in triggering the leukemogenic process when expressed in immature thymocytes.

9. CONCLUSION AND FUTURE DIRECTIONS

Since the isolation of HTLV-1 and its characterization as the etiological agent of ATL, numerous studies have been devoted to unravel the implication of this human retrovirus in the cellular and molecular mechanisms presiding the initiation and development of this disease. Still, 25 years later, as recently underlined, ATL remains a "mystery", despite the observations on the role of viral regulatory proteins in the viral life cycle and/or in the perturbation of cellular homeostasis (7). The present review has focused on the data accumulated in the study of Tax, that have underlined its pivotal and critical activities in modulating the expression of viral and cellular genes. Tax that has been often qualified as an oncoprotein, is mainly implicated in the initiation of a lymphoproliferative process and in the occurrence of a genetic instability, in human primary T cells. Consequently, Tax may be considered as a mitogenic protein able to trigger pre-leukemic events. Remarkably, most of these studies have not addressed the target cell specificity of Tax that may be linked to the leukemogenic activity of HTLV-1. Indeed, leukemias are often considered as the products of the disruption in the differentiation of hematopoietic lineages. Interestingly, we have described that Tax is endowed with the ability to interfere with the development of human T-cells in the thymus. As such, Tax will perturb the temporal activation of T cell-specific genes as well as the coordination of survival, proliferation and development events (Figure 3). The recent introduction of the OP9-

DL1 cells to study the development of T-cells *in vitro* as well as the opportunity to benefit of advances in human-hemato-lymphoid-system mice should provide useful approaches to further investigate the intervention of Tax on thymopoiesis together with the emergence and the fate of pre-leukemic events (62, 63). Finally, the recent identification of the HBZ protein, which at the difference of Tax is always detected in ATL cells, is providing new impetus in the field of HTLV-1 research. Indeed, Tax, by activating transcription factors in T-cell progenitors, is paving the way to the intervention of HBZ during the late stages of the leukemogenic process.

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Abbreviations: ATL, adult T-cell leukaemia; CBP, CREB binding protein; DN, double-negative; DP, double positive; HBZ, HTLV-1 bZip; HSC, hematopoietic stem cells; HTLV-1, human T-cell leukaemia virus type 1; IL-2R, interleukin 2 receptor; ISP, immature single positive; LTR, long terminal repeat; MHC, major histocompatibility complex; SP, single positive; T-ALL, T acute lymphoblastic leukaemia; TCR, T-cell receptor; pTα, pre-TCRα

Key Words: HTLV-1, Tax, ATL, T cell Development, Leukemogenesis, Human Thymocytes, Review

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