Adult Human T Cell Leukemia

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1. Introduction

Human T cell leukemia virus 1 (HTLV-1), the first characterized human retrovirus, has been identified as the causative agent for adult T-cell leukemia/lymphoma (ATLL). This aggressive lymphoid proliferation is associated with a bad prognosis due to the resistance of HTLV-1-infected cells to most classical chemotherapeutic agents.

HTLV-1 is transmitted intravenously, by sexual contact, or through breast-feeding from mother to child, and epidemiological evidence predicts that ATLL development occurs following childhood infection. ATLL exhibits diverse clinical features: the acute, the sub-acute or smoldering, the chronic forms and the ATL lymphoma. In the two most aggressive forms (acute leukemia and lymphoma), the tumor syndrome comprises massive lymphadenopathy, hepatosplenomegaly, lytic bone lesions and multiple visceral lesions with skin and lung infiltration.

HTLV-1 virions infect CD4+ T cells, which represent the main target for HTLV-1 infection in peripheral blood. HTLV-1 associated diseases occur after long periods of virus latency. For years it has been thought that unlike other retroviruses, free virions were poorly infectious. However, a recent study reported that freshly isolated myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) are efficiently and productively infected by cell-free HTLV-1. Furthermore, infected mDC and pDC were able to transfer virions to autologous CD4+ T cells, clearly demonstrating that cell free HTLV-1 can be infectious and target dendritic cells. Innate immune response against HTLV-1 is poorly documented.

We describe here immune response against HTLV-1 and physiological consequences.

2. The Human T cell leukemia virus 1 (HTLV-1)

In 1980 the group of Robert C. Gallo characterized the first human retrovirus, the Human T cell leukemia virus 1 (HTLV-1) (Poiesz, Ruscetti et al., 1980). This virus was recovered from the peripheral blood cells of a patient suffering from adult T-cell leukemia/lymphoma (ATLL). This form of leukemia is a severe T-cell lymphoma proliferation with bad prognostic due to the resistance of HTLV-1-infected cells to most classical chemotherapeutic agents.

We first describe here the epidemiology, the genomic of HTLV-1 virus and its receptor complex.

2.1 HTLV-1 genomic characteristics

HTLV-1 is classified as a complex retrovirus, in the genus delta-retrovirus of the subfamily Orthoretrovirinae. HTLV-1 retrovirus genetic material is composed by a
diploid single strain RNA (Figure 1). The length of the HTLV-1 genome is 9.032 basepair (bp). The group antigens are similar to other retroviruses-(gag), polymerase (pol), and envelope (env) genes are flanked by long terminal repeats (LTR). The LTR consists of U3, R and U5 regions. The U3 region of HTLV-1 controls the virus transcription. It contains essential elements such as the TATA box, which is necessary for viral transcription, a sequence that causes termination and polyadenylation of the RNA messenger and Tax responsive elements (TRE) involved in Tax protein transcription which regulates the transcription of the HTLV-1 provirus. The R region overlaps the 3’ of the U3 region and contains the majority of the Rex response element. The “gag” gene encodes the virus core protein, which is initially synthesized with approximatively molecular weight of 53 kD. During viral maturation this precursor is cleaved to form the matured matrix P19 (MA), the capsid P24 (CA) and the nucleocapsid P15 (NC).

HTLV-1 virions are enveloped into a lipidic membrane and a nucleocapsid that protect the genetic material, the ribonucleic acid (RNA). The lipidic membrane is derived from cellular plasma membrane. The envelope proteins are constituted by the glycoprotein (gp) 21 (Transmembrane subunit, TM) and gp46 (Surface subunit, SU), which are coded by env and are integrated to the lipidic membrane. Matrix protein p19 and p24 are coded by gag and constitute the intern core of viral envelope. The nucleocapsid p15 is also coded by gag and is enveloping the genetic material composed of a diploid single stranded RNA (Figure 1).

Fig. 1. Genomic and proteic structure of HTLV-1 virion.

2.2 HTLV-1 epidemiology
Over the course of more than 30 years, the epidemiology of HTLV-1 has matured. Epidemiologic studies are based on serologic diagnosis by detection of specific antibodies using enzyme-linked immunosorbent assay (ELISA) or by agglutination assay. Thus, the
serologic is confirmed by immunoblot of specific antibodies and polymerase chain reaction (PCR) of genomic DNA from cells of infected patients. The number of HTLV-1 infected people is elevated and the most recent studies estimated at 15-20 millions people infected worldwide (Verdonck, Gonzalez et al., 2007). Epidemiologic studies revealed that density of infected individuals were in Malaysia, Caribbean, Africa (Gabon, Cameroun), South America (Brazil, Guyana, Colombia) and South Japan (Figure 2). However, these numbers are only estimations and probably do not reflect the reality. Indeed, most of infected people are not diagnosed due to the complex and expensive methods of diagnosis. Thus, number of people really infected might be higher especially in developed countries.

Among the HTLV-1-infected population, around 3 to 6% develop the ATLL syndrome. HTLV-1 infection is highly concentrated in some regions especially in South Japan where the prevalence can reach as high as 37% in a selected population (Mueller, Okayama et al., 1996; Yamaguchi, 1994). The reasons for HTLV-1 clustering, the high ubiquity in southwestern Japan but low prevalence in neighboring regions of Korea, China and Eastern Russia are still unknown. For nonendemic geographic regions, HTLV-1 is mainly found in immigrants. The contamination is largely due to sexual contacts with sex workers. However, the prevalence in Europe and North America remains extremly low and does not exceed 0.01% (Proietti, Carneiro-Proietti et al., 2005).

Fig. 2. Origin, spread, and prevalence of HTLV-1.
HTLV-1 is transmitted intravenously, by sexual contact, or through breast-feeding from mother to child, and epidemiological evidence predicts that ATLL development occurs following childhood infection. Mother to child transmission occurs very frequently (around 20%) and is related to mother viral load and prolonged breast-feeding. Indeed, it is now well accepted that HTLV-1 could be transmitted through mother’s milk and is one of the major factor in vertical transmission. Thus, screening of HTLV-1 among blood donors had been extended and breast-feeding among HTLV-1-infected women had been refrained in Japan decreasing vertical transmission.

Finally, it is also possible that HTLV-1 could be transmitted by saliva, which contains HTLV-1 antibodies and proviral DNA. However, there is no clear study demonstrating this way of contamination (Fujino and Nagata, 2000).

Origin and spread hypothesis based on phylogenetic and anthropological data. HTLV-1 originated in African primates and migrated to Asia where it evolved into STLV-1. This early STLV-1 lineage spread to India, Japan, Indonesia, and back to Africa (arrows 1). It crossed the simian–human barrier in Indonesian human beings who migrated to Malesia, resulting in the HTLV-1c subtype (arrows 2). In Africa, STLV-1 evolved through several interspecies transmissions into HTLV-1a, HTLV-1b, and HTLV-1d, HTLV-1e, and HTLV-1f (arrows 3). Because of the slave trade and increased mobility, HTLV-1a was introduced in the New World, Japan, the Middle East, and North Africa (arrows 4). Colours indicate current prevalence estimates based on population surveys and on studies in pregnant women and blood donors. In some countries, HTLV-1 infection is limited to certain population groups or areas. (Verdonck, Gonzalez et al., 2007).

2.3 HTLV-1 receptor complex

For years the HTLV-1 receptor remained unknown and a real mystery. Serious evidences indicated that HTLV-1 entry requires the viral envelope glycoprotein (Env), the surface subunit gp46 and the transmembrane subunit gp21, generated from the cleavage of a precursor gp61. Mutation in any of this proteins or use of blocking antibodies dramatically reduced HTLV-1 infection. Thus, one study demonstrated that glucose transporter GLUT1 was the receptor for HTLV-1 (Manel, Kim et al., 2003). GLUT1 matched all requirement for HTLV-1 entry. GLUT1 is overexpressed by activated T cells, which are targets of HTLV-1. Using small interfering RNA siRNA strategy they demonstrated that downregulation of GLUT1 in cell lines reduced HTLV-1 infection. Furthermore, GLUT1 transfection of GLUT1 negative cells restored HTLV-1 infection, demonstrating that GLUT1 is an essential component of HTLV-1 receptor. More recently, it has been suggested that two other molecules are involved in HTLV-1 infection of target cells: neuropilin 1 (NRP-1) and Heparan Sulfate Proteoglycans (HSPG) (Ghez, Lepelletier et al.).

The Neuropilin-1 was initially identified as a embryonic neurons guidance factor. NRP-1 is a glycoprotein receptor for Semaphorin 3a and VEGF (Vascular endothelial growth factor). It also has been showed that NRP-1 was a key molecule in angiogenesis and is also implicated in the regulation of immune response (Tordjman, Lepelletier et al., 2002). It has been showed that NRP-1 directly binds HTLV-1 virus. The interaction appeared functionnaly relevant since NRP-1 overexpression enhanced syncytium formation in vitro. Furthermore, confocal analysis revealed a strong polarisation of NRP-1 and viral glycoprotein Env at the interface of an infected cell and a target T cell (Ghez, Lepelletier et al., 2006).

HSPG family members are composed of a core protein associated with one or several sulphated polysaccharide side chains (i.e. sulfate glycosaminoglycans). Sulphated
polysaccharide side chains confer to HSPG members electrostatic properties that allow binding to a very large range of proteins, including cytokines, receptors, hormones, chemokines and extracellular matrix proteins. HSPG enhances infection by facilitating the attachment of the particles on target cells and/or allowing their clustering at the cell surface before specific interactions between viral proteins and their receptors that lead to fusion. HSPG had been showed to bind the HIV-1 protein gp120, therefore facilitating HIV-1 infection. Studies demonstrated that inhibition of HSPG dramatically reduced syncitium formation and infection in CD4+ T cells (Lambert, Bouttier et al., 2009). Furthermore, inhibition of HSPG also reduced infection of dendritic cells. Thus, a model involving three partners had been proposed (Figure 3).

Fig. 3. Model for HTLV-1 receptor complex. From Ghez, Lepelletier et al., 2006.

More recently, one study proposed another model for HTLV-1 entry into target cells (Pais-Correia, Sachse et al.). This model proposes that HTLV-1-infected T lymphocytes transiently store viral particles as carbohydrate-rich extracellular assemblies. These carbohydrate assemblies are attached to cell surface and held together by virally-induced extracellular matrix components. This extracellular matrix is made of protein such as collagen, agrin, galectin-3 and tetherin. It should be noted that HSPG is probably a protein of the HTLV-1 extracellular assemblies. This kind of structure was first discovered for bacteria and called “biofilm”. Authors showed that extracellular HTLV-1 biofilms adhere to other cells facilitating viral binding and infection. This form of viral infection is extremely efficient due to high concentration of extracellular viruses on cell surface. Thus, HTLV-1 may use several strategies to infect target cells. However, further studies are needed to clarify the entry of HTLV-1 in patients.
3. The adult T-cell leukemia/lymphoma (ATLL)

Adult T cell leukemia/lymphoma (ATLL) had been shown to be a consequence of HTLV-1 infection (Hinuma, Gotoh et al., 1982). HTLV-1 infection is also responsible for myelopathy/tropical spastic paraparesis (HAM/TSP) (de The, Gazzolo et al., 1985; Gessain, Barin et al., 1985), uveitis and infective dermatitis in children (Manns et al., 1999). We focus in this section on the complex T-cell leukemia/lymphoma induced by HTLV-1 infection.

3.1 HTLV-1 induces T cell leukemia

In the late seventies, a group of leukemia patients with characteristic clinical features and particular geographical distribution were identified. Uchiyama et al proposed adult T cell leukemia (ATL) as a new disease. In 1980 the group of Robert C. Gallo characterized the first human retrovirus responsible for ATL, the Human T cell leukemia virus 1 (HTLV-1) (Poiesz, Ruscetti et al., 1980). Since then, HTLV-1 has been identified as the causative agent for two major syndromes: the adult T-cell leukemia (ATL) (Poiesz, Ruscetti et al., 1981; Robert-Guroff, Nakao et al., 1982) and the HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Jacobson, 1996). More recently, HTLV-1 also has been shown as the causative agent for uveitis and infective dermatitis in children (Manns, Miley et al., 1999). Among the HTLV-1-infected population, around 3 to 6% develop the ATL syndrome. At the present time, it is not known why some infected patients develop ATL and others do not.

3.2 ATL syndrome

The ATL exhibits diverse clinical features and outcome is directly correlated to ATL subtype. The malignancy ranges from a very indolent and slowly progressive lymphoma to a very aggressive and nearly uniformalay lethal proliferative lymphoma. The incidence of ATL is estimated to be 61/100,000 HTLV-1 carriers, and the crude lifetime risk for developing ATL is 7.3% for males and 3.8% for females. Four types of ATL had been described based on the number of abnormal T cells in peripheral blood, tumor lesions in organs, serum lactic acid dehydrogenase (LDH) level and clinical course: the sub-acute or smoldering (5%), the acute (55%), the chronic forms (20%) and the ATL lymphoma (20%). Patients with smoldering ATL exhibit skin lesions, minimal lymph node enlargement and few leukemic cells in blood. Chronic ATL is characterized by mild symptoms and longer clinical course. In the two most aggressive forms (acute leukemia and lymphoma), the tumor syndrome comprises massive lymphadenopathy, hepatosplenomegaly, lytic bone lesions and multiple visceral lesions with skin and lung infiltration. Acute ATL is also characterized by general malaise, fever, cough, high lactate deshydrogenase (LDH) serum levels, and appearance of multilobulated nuclei leukemic cells. This aggressive lymphoid proliferation is associated with a bad prognosis due to the resistance of HTLV-1-infected cells to most classical chemotherapeutic agents. The cancer is thought to be due to the pro-oncogenic effect of viral DNA incorporated into host lymphocytes DNA, and chronic stimulation of the lymphocytes at the cytokine level may play a role in development of malignancy. The time between infection and onset of cancer also varies geographically. It is believed to be about sixty years in Japan, and less than forty years in the Caribbean.

3.3 The ATL cell

The ATL cell is easily characterized by histological and/or cytological infiltration by flower cells (Matsuoka, 2005) that are malignant activated lymphocytes with convoluted nuclei and
basophilic cytoplasm, a multilobed nucleus with a flower shape (Figure 4). ATL cells express most of T cell markers (CD2, CD3, CD4, CD45RO) and more rarely CD8. However, ATL cells also express the alpha chain of IL-2 receptor, CD25, and T cell activation markers such as major histocompatibility complex HLA-DR and HLA-DQ. ATL is well known to infiltrate various organs and tissues, such as the skin, lungs, liver, gastrointestinal tract, central nervous system and bone. This infiltrative tendency of leukemic cells is possibly attributable to the expression of various surface molecules, such as chemokine receptors and adhesion molecules. Skin-homing memory T-cells uniformly express CCR4, and its ligands are thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC). CCR4 is expressed on most ATL cells. In addition, TARC and MDC are expressed in skin lesions in ATL patients. Thus, CCR4 expression should be implicated in the skin infiltration (Yoshie, 2005). On the other hand, CCR7 expression is associated with lymph node involvement (Kohno, Moriuchi et al., 2000). OX40 is a member of the tumor necrosis factor family and was reported to be expressed on ATL cells (Imura, Hori et al., 1997; Kunitomi, Hori et al., 2002).

Fig. 4. Typical "flower cell" in the peripheral blood of an acute ATL patient observed on microscope. In the peripheral blood of an acute ATL patient, leukemic cells with multilobulated nuclei (Matsuoka, 2005).

3.4 Treatment of ATL
Adult T cell leukemia is an aggressive malignant disease that results from HTLV-1 infection. The prognostic is directly correlated to the ATL subtype. Since ATL is a neoplasm of mature T cells, it has been treated with chemotherapies for non-Hodgkin's lymphoma. Conventional chemotherapy (LSG15) had only transient effect and the prognostic remains poor. The median survival time (MST) never exceeded 10 month. Therefore, new therapeutic strategies were tested to adapt treatment to ATL subtype reviewed by Kimiharu Uozumi (Uozumi). New strategies can be divided in 3 main groups: chemical anti-tumor agents, monoclonal antibodies and vaccination. Among new chemical anti-tumor agents the combined use of anti-retroviral drug AZT and recombinant interferon alpha (IFN-α) showed promising results (Hermine, Bouscary et al., 1995). The MST was increased in most patients and this therapy constitutes one of the most efficient at the present time.
Similarly the combined use of arsenic trioxid and interferon alpha exhibits an anti-leukemia effect in very poor prognosis ATL patients despite a significant toxicity (Hermine, Dombret et al., 2004).

Treatment using monoclonal antibodies and recombinant cytokines are also very promising. We will describe later in section 4 the use of TNF-related Apoptosis Inducing Ligand (TRAIL), a Tumor Necrosis Factor (TNF) superfamily member (Wiley, Schooley et al., 1995), as a new therapeutical strategy to induce ATL cells apoptosis.

4. HTLV-1 and immune response

HTLV-1 is a retrovirus and therefore is recognised by the immune system as foreign agent. Immune system is activated after infection and produce specific anti-HTL-1 antibodies. Most of immune cells respond to HTLV-1 virions. However, because symptoms occur after a long period of latency it is extremely hard to study acute infection. Thus, most of immunologic studies are performed using samples from patients infected since several years. We review in this section the interactions between immune cells and HTLV-1 and provide some new features concerning innate immune response.

4.1 Immune cell activation by HTLV-1

HTLV-1 like HIV-1 is a retrovirus and induces a chronic disease. Although a large number of studies have indicated that initial virus infection involves majority viral invasion of CD4+ T cells, which represent an important target for HTLV-1 infection in the peripheral blood, additional evidence has demonstrated that HTLV-1 can infect several additional cellular compartments in vivo, including CD8+ T lymphocytes, monocytes, dendritic cells, B lymphocytes residing in the peripheral blood and lymphoid organs or resident central nervous system (CNS) astrocytes (Koyanagi, Itoyama et al., 1993; Macatonia, Cruickshank et al., 1992; Nagai, Kubota et al., 2001; Richardson, Edwards et al., 1990). Transient phase of reverse transcription of viral RNA is followed by a persistent phase of clonal expansion within the CD4+ and CD8+ T cell populations (Mansky, 2000; Mortreux, Leclercq et al., 2001). Very little viral gene expression and low amounts of infectious virus production of HTLV-1 infected monocyte/macrophage lineage and dendritic cells are likely attributable to their postmitotic status and relatively short lifetime (Banchereau and Steinman, 1998; Valledor, Borras et al., 1998).

This differential viral gene expression between T and dendritic cells depending of viral clonal expansion and expression drives the HTLV-1 immune response. Although dendritic cells have a low level of viral gene expression, recent evidence has suggested that HTLV-1-infected dendritic cells exhibit an enhance capacity to stimulate antigen-specific T cell activation (Makino, Shimokubo et al., 1999). Furthermore, the Th1-type cytokines IL-1β, interferon-γ (IFN-γ), and TNF-α were overexpressed in asymptomatic carriers and patients with HAM/TSP, while the Th2/Th3-type cytokine transformin growth factor (TGF-β) was overexpressed in patients with ATL (Tendler, Greenberg et al., 1991). Several events may lead to stimulation of a Th1 or a Th2/Th3 T cell response besides the subset of dendritic cells (DC1 and DC2) that are first encountered antigen, or depending of the pathogen, recognition receptors and site of exposure (Pulendran, Palucka et al., 2001). Furthermore, the type of T cell response is dependent of both DC ontogeny, but also of the dendritic cell activating stimulus (Grabbe, Kampgen et al., 2000). Consequently, the initial route of
infection determines the preferential infection of dendritic cell subsets but these consequences of event remain unknown. Furthermore, the proportion of blood and secondary lymphoid organs HTLV-1+ DC is proportional to the total proviral DNA load in the blood, providing a correlation of proviral DNA load and the frequency of effector/memory Tax- CD8+ T cells (Nagai, Kubota et al., 2001).

HTLV Tax oncogene may be released and act as a cytokine on neighboring cells in the CNS inducing NF-kB nuclear localization and immunoglobulin κ light chain, IL-2Ra, IL-1b, IL-6, TNF-α, and TNF-β expression (Lindholm et al., 1990; Lindholm et al., 1992; Marriott et al., 1992; Dhib-Jalbut et al., 1994). The cytokine like effects of Tax may induce a signaling cascade by binding to a specific cell surface receptor (DC1 and/or DC2 NP-1). Interestingly, one of the members of the interferon regulatory factor (IRF) family – IRF-4 – was shown to be highly expressed in cells derived from patients with ATL and in HTLV-1 infected cell lines (Imaizumi, Kohno et al., 2001; Mamane, Grandvaux et al., 2002; Mamane, Loignon et al., 2005; Sharma, Grandvaux et al., 2002; Sharma, Mamane et al., 2000; Yamagata et al., 1996). A detailed analysis of IRF-4 has implicated the viral Tax protein in mediating activation of the Sp1, NF-kB and NF-AT pathways leading to a feed back loop mediated by Tax (Grumont and Gerondakis, 2000; Sharma, Grandvaux et al., 2002; Sharma, Mamane et al., 2000).

4.2 ATL and TRAIL-induced apoptosis

Induction of tumor cell death by apoptotic molecules is one of strategy that could be used to selectively reduce cancer cell proliferation without damaging normal tissue. The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a TNF superfamily member, has been shown to induce apoptosis of the vast majority of tumor cell lines (Wiley, Schooley et al., 1995). TRAIL-induced apoptosis is finely regulated by the expression of two groups of receptors. Three receptors do not induce apoptosis (Decoy Receptors, DcR) and two activate apoptosis of target cells (Death Receptor 4 and 5, DR4, DR5) (Sheikh, Burns et al., 1998; Sheridan, Marsters et al., 1997; Wu, Burns et al., 1997). The two biologically active forms of TRAIL, membrane-bound (mTRAIL) and soluble TRAIL (sTRAIL), are regulated by type I interferon (interferon-alpha and beta: IFN-α and IFN-β)(Ehrlich, Infante-Duarte et al., 2003; Sato, Hida et al., 2001; Tecchio, Huber et al., 2004). TRAIL active form consist of a trimer stabilized by a zinc molecule. TRAIL is secreted by leukocytes, including T lymphocytes (Kayagaki, Yamaguchi et al., 1999), natural killer cells (Smyth, Cretney et al., 2001), dendritic cells (Vidalain, Azocar et al., 2000; Vidalain, Azocar et al., 2001), monocytes and macrophages (Herbeuval, Lambert et al., 2003). TRAIL can activate both intrinsic or extrinsic apoptosis pathway. DR4 and DR5 induced apoptosis through the formation of a death inducing signaling complex (DISC) containing the death receptor, adaptor proteins such as Fas-associated death domain (FADD), and initiator caspases such as pro-caspase-8 or pro-caspase-10 (Bellail, Tse et al., 2009; Jin, Kurakin et al., 2004; Walczak and Haas, 2008). Consequently, pro-caspase-8 or pro-caspase-10 are activated by autoproteolytic processing, which then cleave and activate downstream effector caspases (Gomez-Benito, Martinez-Lorenzo et al., 2007), such as caspase-3 (extrinsic pathway). Additionally, the Bcl-2-interacting protein Bid is also cleaved by caspase-8. Truncated-Bid causes the loss of mitochondrial membrane potential and caspase-9 cleavage, resulting in apoptosis. Very little is known concerning death and decoy receptors regulation. Among these receptors, death
receptor is the most studied, and authors showed that DR5 transcription is regulated (at least partially) by the protooncogene p53 (Sheikh, Burns et al., 1998; Wu, Burns et al., 1997). It should be noticed that TRAIL death receptors 4 and 5 not only induce apoptosis but may also play a crucial role in inflammatory responses (Collison, Foster et al., 2009). Figure 5 illustrates TRAIL pathway and regulation.

TRAIL is a very promising candidate for cancer treatment due to its sophisticated way of inducing apoptosis. While the vast majority of normal cells express decoy receptors and are therefore protected from TRAIL-mediated apoptosis, tumor cells generally express death receptors. Indeed, TRAIL induces apoptosis in human tumor cell lines (Griffith, Chin et al., 1998) but not in normal cells (Gura, 1997). TRAIL also induces apoptosis of infected cells. For example, plasma TRAIL has been reported to be an early pathogenic marker in acute HIV-1 infection and is correlated to viral load in chronic disease (Gasper-Smith, Crossman et al., 2008; Herbeuval, Nilsson et al., 2009). HIV-1 upregulates DR5 expression on the membrane of CD4+ T cells in vitro (Herbeuval, Boasso et al., 2005; Herbeuval, Grivel et al., 2005) making them prone to TRAIL-mediated apoptosis (Lichtner, Maranon et al., 2004). Furthermore, the percentage of CD4+ T cells co-expressing TRAIL and DR5 are elevated in the blood of viremic progressors (Herbeuval, Grivel et al., 2005). Thus, TRAIL does not exhibit cytotoxic effects on normal cells and tissues and is potentially efficient to eradicate a large panel of cancer cells. Several clinical trial are currently evaluating TRAIL anti tumor effect, alone or in combination with other chemotherapeutic drugs.

Thus, it remained pertinent to determine whether ATL cells were sensitive to TRAIL-mediated apoptosis. One study characterized the sensitivity of ATL cells to TRAIL cytotoxicity. Authors tested several cell lines and also primary cells from both chronic and acute ATL. Unfortunately, the vast majority of primary ATL cells or cell lines appears to be resistant to TRAIL induced cell death (Matsuda, Almasan et al., 2005). This resistance was due to multiple parameters, including the lack of DR4 and DR5 expression, abrogation of death signal upstream caspase-8, attenuation of both extrinsic and intrinsic apoptotic pathways. More recently, it has been shown that the resistance upstream caspase 8 was due to an over expression of the cellular caspase-8 (FLICE)-inhibitory protein (c-FLIP) that blocks caspase recruitment and apoptosis. However, other study show that ATL cells might be sensitive to TRAIL-induced apoptosis (Hasegawa, Yamada et al., 2005), therefore TRAIL effect in ATL should be clarified.

Surprisingly, most of ATL cells expressed TRAIL on their surface. This finding suggested that constitutive expression of TRAIL would participate in the development of TRAIL-resistant clones observed in patients. The natural resistance of ATL cells would have excluded the use of TRAIL as therapeutic agent. However, a recent study demonstrated that the herbal compound Rocaglamide restores TRAIL sensibility in ATL cells. Indeed, Rocaglamide induces suppression of c-FLIP expression in ATL cells that sensitizes these cells to TRAIL-mediated apoptosis. Authors suggest the use of Rocaglamide as an adjuvant to TRAIL as new therapeutic strategies against HTLV-1-mediated ATL (Bleumink, Kohler et al.). It has also been observed that the use of a combination of a p53 activator, Nutlin-3a, and TRAIL synergized to induce ATL cell apoptosis (Hasegawa, Yamada et al., 2009). This could be explained by the fact that p53 regulates TRAIL death receptor 5 on cell surface. Thus, Nutlin-3a treated ATL cells would express DR5 and then become sensitive to TRAIL-mediated apoptosis.

Therefore, the understanding of ATL sensitivity to TRAIL-mediated apoptosis appears to be crucial to develop new therapeutic options.
Fig. 5. TRAIL apoptotic pathway. Membrane (mTRAIL) or soluble TRAIL (sTRAIL) bind to 3 decoy receptors (DcR1, DcR2 and OPG) and 2 death receptors (DR4 and DR5) which activate the caspase pathway leading to apoptosis.

4.3 Myeloid dendritic cells and HTLV-1 infection
HTLV-1 targets CD4+ T cells which represent an important target for HTLV-1 infection in the peripheral blood. However, there are some additional evidence that showed that HTLV-1 can also infect including CD8+ T lymphocytes, monocytes, B lymphocytes, astrocytes (Richardson, Edwards et al., 1990) and dendritic cells (DC) in vivo. Myeloid dendritic cells do not exhibit high viral gene expression, but recent work suggested that HTLV-1-infected dendritic cells show better capacity to stimulate antigen-specific T cell activation (Makino, Shimokubo et al., 1999). Moreover, the proportion of lymphoid organs containing HTLV-1 positive dendritic cells is proportional to the total proviral DNA load in the blood, providing a correlation of proviral DNA load and the frequency of effector/memory Tax-CD8+ T cells (Nagai, Kubota et al., 2001).

More recently, findings demonstrated a central role to myeloid dendritic (mDC) and plasmacytoid dendritic cells (pDC) in HTLV-1 infection. For years it has been thought that unlike other retroviruses such as HIV-1, free virions were poorly infectious (Donegan, Lee et al., 1994). However, a recent study reported that freshly isolated mDC and pDC are efficiently and productively infected by cell-free HTLV-1 (Jones, Petrow-Sadowski et al.,...
Furthermore, infected mDC and pDC were able to transfer virions to autologous CD4+ T cells, clearly demonstrating that cell free HTLV-1 can be infectious and target dendritic cells (Jones, Petrow-Sadowski et al., 2008).

5. HTLV-1 and plasmacytoid dendritic cell response

Plasmacytoid dendritic cells were discovered in 1997 as professional IFN-alpha producers and innate immune cells (Grouard, Rissoan et al., 1997). These cells are rare but play a central role in host defense against viruses and bacteria by producing cytokines and antiviral factors. The role of pDC in HTLV-1 infection remained unknown until recent years probably because of the extreme difficulty of studying HTLV-1 acute infection. We describe here recent data providing some new features in the understanding of HTLV-1 innate immune response.

5.1 The Plasmacytoid dendritic cell (pDC)

PDC are cells of hemopoietic origin that are found at steady state in the blood, thymus and peripheral lymphoid tissues. Early studies described pDC as being oval-shaped with typical plasmacytoid morphology. The ability of plasmacytoid-derived DC (also named DC2) to induce a Th2 differentiation of naïve CD4 T cells formed the basis for the concept of type 1 and type 2 DC (Review Nat immunol, 2001). The role of these DC in mouse and human was studied in different models and is not completely elucidated (Liu, 2005). A little later, it was shown that pDC were specialized in the production of type I IFN (Siegal, Kadowaki et al., 1999). They are the principal source of type I IFN in human blood and very rapidly produce all type I IFN isoforms in response to microbial stimuli, such as virus (Cella, Jarrossay et al., 1999; Siegal, Kadowaki et al., 1999), CpG-containing oligonucleotides (Kadowaki, Antonenko et al., 2000), or the synthetic molecules imidazoquinolines (Gibson, Lindh et al., 2002). PDC-derived type I IFN has direct antiviral activity against a variety of virus, including HIV, and has important adjuvant functions on other immune cell-types, such as NK cells, T cells, macrophages and DC. Thus, pDC activation triggers a dual type of response: type I IFN production and DC differentiation (Colonna, Trinchieri et al., 2004; Yang, Lian et al., 2005).

PDC and plasmacytoid-derived DC express the Toll-Like receptor TLR7 and TLR9 (Jarrossay, Napolitani et al., 2001; Kadowaki and Liu, 2002) and respond to their respective ligands, imidazoquinolines (Hemmi, Kaisho et al., 2002) and single strand RNA (Diebold, Kaisho et al., 2004; Heil, Hemmi et al., 2004; Lund, Alexopoulou et al., 2004) for TLR7, CpG-containing oligonucleotides (Hemmi, Takeuchi et al., 2000) and DNA viruses (Lund, Sato et al., 2003) for TLR9. They do not express TLR2, TLR3 and TLR4, and do not respond to such ligands as peptidoglycan, LPS (lipopolysaccharides) or double-stranded RNA (Jarrossay, Napolitani et al., 2001; Kadowaki and Liu, 2002). Activation of pDC through TLR7 and TLR9 can trigger both types of response, including large quantities of type I IFN production and DC differentiation (Liu, 2005). Synthetic CpG-containing oligonucleotides of the types A and B (CpG-A, CpG-B) selectively induce type I IFN production and DC differentiation, respectively (Duramad, Fearon et al., 2005) while some viral stimuli, such as influenza virus (Flu), herpes simplex virus (HSV) or CpG-C can induce simultaneously both responses (Liu, 2005). Two factors seem to be key for the induction of large quantities of type I IFN in pDC: 1) the ability of the TLR ligands to bind its receptor in the early endosomal compartments (Guiducci, Ott et al., 2006; Honda, Ohba et al., 2005); 2) the...
phosphorylation and nuclear translocation of the transcription factor IRF-7 (Honda, Yanai et al., 2005). This last step was shown to depend on the kinases IRAK-1 (Uematsu, Sato et al., 2005) and IκB kinase-α (IKK-α) (Hoshino, Sugiyama et al., 2006) in mouse pDC. It has been recently shown that the PI3-kinase pathway was critical to control the nuclear translocation of IRF-7 and the subsequent production of type I IFN (Guiducci, Ghirelli et al., 2008). At the present time, it is not known whether additional molecular pathways are involved and modulated HTLV-1 diseases.

PDC express a panel of surface receptors but their function remain largely unknown. The best characterized is the lectin BDCA-2 (Blood dendritic cell antigen-2) (Dzionek, Inagaki et al., 2002; Dzionek, Sohma et al., 2001). BDCA-2 mediates antigen uptake and inhibits pDC production of type 1 IFN induced by influenza virus (Dzionek, Sohma et al., 2001). This inhibition is mediated by the induction of a B cell receptor-like signaling cascade (Cao, Zhang et al., 2007). Neuropilin 1 (NRP1), also called BDCA-4, is another surface receptor constitutively expressed at high levels on human pDC. NRP1 is involved in the interaction between myeloid DC and T cells within the immune synapse (Tordjman, Lepelletier et al., 2002). However, its role in pDC function remains unknown. Recently, we have shown that NRP1 was a coreceptor for the HTLV-1 virus and might be involved in viral entry (Ghez, Lepelletier et al., 2006). Thus HTLV-1 could provide a link between the molecular pathways downstream of NRP1 and the physiopathology of pDC in HTLV-related diseases.

There is increasing evidence that pDC are involved in several disease settings. They were observed in situ in a variety of pathological conditions, such as HPV-related cervical cancer, skin melanoma (Salio, Cella et al., 2003), psoriasis (Nestle, Conrad et al., 2005) or allergic contact dermatitis (Bangert, Friedl et al., 2003) and in the nasal mucosa as early as 6 hours after allergen challenge, suggesting an active recruitment of blood pDC at the site of inflammation. Moreover, a dysregulated TLR-induced IFN response has been linked to autoimmune diseases (Colonna, 2006; Marshak-Rothstein, 2006), particularly lupus erythematosus and psoriasis (Nestle, Conrad et al., 2005).

5.2 pDC and HTLV-1 infection

Three molecules have been characterized for HTLV-1 entry into cells, heparin sulfate proteoglycans (HSPG) (Jones, Petrow-Sadowski et al., 2005) and NRP-1 (also called BDCA-4) for the initial virus binding to target cells (Ghez, Lepelletier et al., 2006), and glucose transporter 1 (GLUT-1) for the post-attachment and the viral fusion (Manel, Kim et al., 2003; Takenouchi, Jones et al., 2007). Interestingly, NRP-1 is expressed by mDC and T cells but cells expressing the highest level of BDCA-4 in blood are pDC (Grouard, Rissoan et al., 1997; Siegal, Kadowaki et al., 1999), strongly suggesting that HTLV-1 could interact with pDC. Nevertheless, HTLV-1-induced immune response by professional “sentinel” pDC has not been reported. Viral activation of pDC can be regulated by either of two Toll-like Receptors (TLR), TLR7 or TLR9, which are considered to be the receptors that human pDC use for recognition of RNA/retroviruses and DNA, respectively. HIV-activated pDC were recently reported to express the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) (Chaperot, Blum et al., 2006; Hardy, Graham et al., 2007; Stary, Klein et al., 2009). TRAIL has been shown to induce apoptosis of cancer (Herbeuval, Lambert et al., 2003; Walczak, Miller et al., 1999) and infected cells expressing death receptor-4 or -5 (DR4, DR5). We recently demonstrated that HTLV-1 stimulated pDC expressed TRAIL and acquired cytotoxic activity, transforming them into a new subset of killer innate immune cells, which
may play a central role in viral immunopathogenesis and tumor development (Hardy, Graham et al., 2007).

The role of pDC in HTLV-1 infection was unknown until Jones et al reported that freshly purified mDC and pDC could be productively infected by HTLV-1 free viruses (Jones, Petrow-Sadowski et al., 2008). Authors clearly demonstrated that infected pDC and mDC could also infect CD4+ T cells in vitro. These findings were major discovery for two reasons: first, they showed that, unlike researcher thought for years, free particles of HTLV-1 could directly infect T cells, and secondly because it was the first demonstration of pDC-HTLV-1 interaction.

However, the infection of target cells by HTLV-1 is not totally understood and need to be clarified. HTLV-1 infection is a sequential process that potentially involves the recruitment of at least three molecules.

5.3 pDC activation by cell free HTLV-1 virions

Due to obvious evidences that pDC and HTLV-1 could interact, we decided to study pDC response against HTLV-1. The first parameter we tested was the IFN-α production, which is characteristic of the innate immune response. Our first results were disappointing. Using supernatants from chronically HTLV-1-infected cell lines we stimulated pDC in vitro. The IFN-α produced by pDC exposed to supernatants from MT-2 cell line remained very low compared to Influenza A (Flu) stimulation. The difference between the two stimulation was the purity of the viruses: Flu was a purified virus while HTLV-1 stimulation was made from supernatants. Thus, we decided to purify HTLV-1 by ultracentrifugation. Pellets were collected and the quantity of viruses was determined using p19 ELISA. Thus, we could calculate the concentration of purified HTLV-1 and use a large range of viral concentration to stimulate pDC. Therefore, we showed that purified cell free HTLV-1 particles could induce massive IFN-α by pDC, similarly to Influenza A virus (Flu) or HIV stimulation. For the first time, we clearly demonstrated that free HTLV-1 particles, as other retroviruses, could generate an IFN-α response by pDC (Colisson, Barblu et al, 2010). We also tested IL-10 and TNF-α production by HTLV-1-exposed pDC and found high levels of IL-10 and TNF-α production.

We and others reported that Flu or HIV-1 activation of pDC resulted in cytokine production but also activation markers (CD40, HLADR), maturation markers (CD80, CD86) and migration marker CCR7 (Beignon, McKenna et al., 2005; Chaperot, Blum et al., 2006; Fonteneau, Larsson et al., 2004). We found that HTLV-1, like other retroviruses, induced activation and maturation marker expression by pDC. However, it remained unclear whether the lymphoid migration marker CCR7 was expressed by pDC after HTLV-1 exposure. This might have essential consequences in immunopathogenesis. Indeed, HIV-1 induces migration of pDC from the blood to lymphoid organs by upregulating CCR7 expression on cell surface. Thus, activated pDC migrate to tonsils and other lymphoid organs and participate to CD4+ T cell depletion in tissues (Stary, Klein et al., 2009). We observed that CCR7 was not or weakly expressed by pDC after HTLV-1 exposure. Consequently, we could imagine that pDC do not migrate to lymphoid tissues in HTLV-1 infected patients, in contrast to HIV-1 patients. Further studies are needed to better characterized in vitro and in vivo CCR7 expression and pDC migration in patients.

We next wanted to better characterize pDC activation by HTLV-1. We previously reported that HIV-1 induced pDC transformation into TRAIL-expressing pDC, which were able to induce apoptosis of CD4+ T cells expressing DR5 (Hardy, Graham et al., 2007). This new
subset of killer cells was called Interferon-producing Killer pDC (ie IKpDC). Using cell free purified HTLV-1 particles, we stimulated isolated pDC from healthy donors and cells were analyzed using three dimensional (3D) microscopy. Microscopy revealed some surprising results. We found high levels of TRAIL in non activated pDC. This result was not expected as it has never been observed before. However, it was not clear whether TRAIL was located on membrane or in cytoplasm. Thus, using the ImageJ tool "3D interactive surface plot", we demonstrated that TRAIL was located in the cytoplasm of resting pDC (Colisson, Barblu et al.). In contrast, HTLV-1 stimulated pDC showed a relocalization of TRAIL from cytoplasm to plasma membrane (Figure 6). HTLV-1 exposure induced a relocalization of intracellular stock of TRAIL to the membrane, conferring a killer activity to pDC. Surprisingly, we did not detect HTLV-1 viruses in pDC. However, chloroquine treated pDC revealed some HTLV-1 particles in cytoplasm. In fact, this latest result provides some indication concerning the pathway by which HTLV-1 particles activate pDC.

**Fig. 6.** 3D microscopy of HTLV-1 activated pDC. 3D interactive surface plot was used to precisely delimitated TRAIL in pDC. Upper picture show intracellular TRAIL (green) inside pDC plasma membrane. Bottom picture shows TRAIL relocalization to the membrane that appears green in HTLV-1 exposed pDC. HTLV-1 viruses (red) binds to plasma cell membrane. Adapated from Colisson, Barblu et al 2010.

### 5.4 HTLV-1 activated endocytosis pathway in pDC

Because pDC express high levels of NRP-1, which is a member of the HTLV-1 complex receptor, they may be productively infected by HTLV-1. However, pDC could also activate the endocytosis pathway under viral exposure. Endocytosis pathway is characterized by formation of endosomes in which pH get low activating multiple protease. Viral particles are degraded by low pH proteases and genetic material is released into the vesicles. Thus,
viral RNA or DNA activate their respective receptors TLR7 or TLR9. Chloroquine inhibits endocytosis by reducing pH acidification in endosomes. We demonstrated using chloroquine and TLR7 inhibitor that HTLV-1 activated the endocytosis pathway in pDC as demonstrated for other retrovirus like HIV-1 (Beignon, McKenna et al., 2005; Hardy, Graham et al., 2007). Viral particles, after initial binding to HTLV-1 receptor complex, entered into the endosome, which became pH low. This acidification activates endosomal protease that destroyed virus envelop and capsid, leading to single strand RNA (ssRNA) release into the vesicles. This viral ssRNA activates TLR7, which in turn recrutes the adaptor protein MyD88, a central molecule in most of TLR-dependent. The recruitment of MyD88 starts a cascade of activation leading to IFN-α production (due to the recruitment of Interferon Regulatory factor 7, IRF7). We also showed that TRAIL expression, activation markers (CD40, HLADR) and maturation markers (CD80, CD86) were regulated by TLR7 activation in pDC. These results place TLR7 as the central molecule of HTLV-1-induced pDC response (Figure 7). Thus, endocytosis seems to be the major pathway involved in pDC activation by HTLV-1. However, it should be noticed that our findings do not exclude the possibility that pDC could get productively infected. Our study focused on short time experiments that did not allow us to detect newly synthesized viruses. Jones et al showed that coculture of mDC and pDC with HTLV-1 could induce CD4+ T cell infection, while free viruses alone could not infect T cells (Jones, Petrow-Sadowski et al., 2008). Further experiments are needed to determine what is the proportion of infected pDC versus activated IKpDC.

**Fig. 7. Transformation of pDC into IKpDC by HTLV-1.**
6. Conclusion

Adult T cell leukemia induced by HTLV-1 infection exhibits diverse clinical features. The outcome is directly correlated to ATL subtype, that could range from a very indolent and slowly progressive lymphoma to a very aggressive and nearly uniformly lethal proliferative lymphoma. Thus, knowledge about HTLV-1 infection and propagation remains essential to better understand pathogenesis consequences.

An important challenge would be to link the pDC phenotype to the different HTLV-1 associated pathologies (ATLL). It would be interesting to determine whether IKpDC persist during chronic infection in order to generate new HTLV-1 progression markers. The characterization of IKpDC in vivo opens new area of dendritic cells research in HTLV-1 and other retrovirus-induced immunopathogenesis and in tumor cell biology. Considered together, our data highlight a dual role for pDC in HTLV-1 disease. pDC that become infected may participate in viral spread in the host (Jones, Petrow-Sadowski et al., 2008) and concomitantly express TRAIL, which may select the transformed CD4+ T cell clone, leading to ATLL years later. In this context, it will be of great interest to test TRAIL sensitivity of the persistent clones after HTLV-1 infection that may subsequently be transformed to lymphoma/leukemia. Thus, pDC investigation in HTLV-1 disease will be crucial for understanding complex HTLV-1-associated pathologies. However, detection of primary infection in humans is currently not feasible due to the high latency of HTLV-1 virus before disease symptoms appearance. An alternative way to characterize and understand the early steps of HTLV-1 infection is the development of the pathogenic simian model (STLV-1). However, in addition to selection of TRAIL-resistant clones, one could hypothesize that similar to HIV-1 infection, pDC may participate in and contribute to the immune suppression that occurs in ATLL.

HTLV-1 free particles generate an immune response by professional virus “sentinel” pDC. We then identify and describe the mechanism by which purified HTLV-1 virions stimulate pDC and transform them into functional killer cells. We show that pDC response and activation to HTLV-1 is strictly virus-dose dependent. Finally, purified HTLV-1 particles induced TLR7-mediated relocalization of intracellular TRAIL to the pDC membrane. In conclusion, the physiological function of pDC during the different stages of HTLV-1 infection will represent a new field of investigation and may lead to new therapeutic strategies.

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8. References


attachment and entry of human T-cell leukemia virus type 1 virions into CD4+ T


Kadowaki, N. and Y. J. Liu (2002). "Natural type I interferon-producing cells as a link
between innate and adaptive immunity." *Hum Immunol* 63 (12): 1126-32

Kayagaki, N., N. Yamaguchi, et al. (1999). "Involvement of TNF-related apoptosis-inducing

Kohno, T., R. Moriuchi, et al. (2000). "Identification of genes associated with the progression
of adult T cell leukemia (ATL)." *Jpn J Cancer Res* 91 (11): 1103-10


resistant to Fas-induced apoptosis." *Int J Hematol* 76 (3): 260-6

Lambert, S., M. Bouttier, et al. (2009). "HTLV-1 uses HSPG and neuropilin 1 for entry by
molecular mimicry of VEGF165." *Blood*

death in infected and uninfected primary CD4 T lymphocytes." *AIDS Res Hum Retroviruses* 20 (2): 175-82


spastic paraparesis are infected with HTLV-1 and stimulate autologous lymphocyte
proliferation." *AIDS Res Hum Retroviruses* 8 (9): 1699-706

(HTLV-1)-infected dendritic cells in the development of HTLV-1-associated

leukemia virus-1 infection." *Oncogene* 21 (44): 6751-65

Mamane, Y., M. Loignon, et al. (2005). "Repression of DNA repair mechanisms in IRF-4-
expressing and HTLV-1-infected T lymphocytes." *J Interferon Cytokine Res* 25 (1): 43-51


The purpose of this book is to provide a comprehensive review of the scientific advances in T-cell malignancies and to highlight the most relevant findings that will help the reader understand both basic mechanisms of the disease and future directions that are likely to lead to novel therapies. In order to assure a thorough approach to these problems, contributors include basic scientists, translational researchers and clinicians who are experts in this field. Thus, the target audience for this book includes both basic scientists who will use this book as a review of the advances in our fundamental knowledge of the molecular mechanisms of T-cell malignancies, as well as clinicians who will use this book as a tool to understand rationales for the development of novel treatments for these diseases.

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