Differential transforming activity of the retroviral Tax oncoproteins in human T lymphocytes

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Human T cell leukemia virus type 1 and type 2 (HTLV-1 and -2) are two closely related retroviruses. HTLV-1 causes adult T cell leukemia and lymphoma, whereas HTLV-2 infection is not etiologically linked to human disease. The viral genomes of HTLV-1 and -2 encode highly homologous transforming proteins, Tax-1 and Tax-2, respectively. Tax-1 is thought to play a central role in transforming CD4+ T lymphocytes. Expression of Tax-1 is crucial for promoting survival and proliferation of virally infected human T lymphocytes and is necessary for initiating HTLV-1-mediated oncogenesis. In transgenic mice and humanized mouse model, Tax-1 has proven to be leukemogenic. Although Tax-1 is able to efficiently transform rodent fibroblasts and to induce lymphoma in mouse model, it rarely transforms primary human CD4+ T lymphocytes. In contrast, Tax-2 efficiently immortalizes human CD4+ T cells though it exhibits a lower transforming activity in rodent cells as compared to Tax-1. We here discuss our recent observation and views on the differential transforming activity of Tax-1 and Tax-2 in human T cells.

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immortalization or transformation during HTLV-1 infection of T cells, a variety of cellular oncogenic alterations are expected to occur. Thus, studying these processes will provide crucial insights into the pathogenesis of ATL.

We previously hypothesized that in the absence of other viral proteins, Tax-1 is sufficient to immortalize human mature CD4+ T cells while Tax-2 preferentially immortalizes CD8+ T cells. We unexpectedly found that both Tax-1 and Tax-2 failed to immortalize human primary CD4+ T cells, and Tax-2 was able to immortalize human CD4+ T cells more efficiently than Tax-1 in the study with including 12 healthy blood donors. A similar finding was reported later (Inui et al., 2011). In addition, a majority of Tax-2-immortalized CD4+ T cell lines grew in vitro at the growth rate similar to some lymphoblastic leukemia cells (Ren et al., 2012). In contrast, Tax-1-immortalized CD4+ T cells were slow growing and exhibited spontaneous cell death at normal culture conditions (Ren et al., unpublished data). These experimental results were unlikely caused by technical variation because Tax-1 and Tax-2, in which their expressions were driven from human elongation factor promoter, were introduced into human primary T cells via VSV-G pseudotyped lentiviruses at similar efficiency.

Tax-2 appears to be more oncogenic than Tax-1 in primary CD4+ T cells when they are expressed alone, however Tax-1 is clearly oncogenic in CD4+ T cells in the context of an intact proviral clone and other expressed viral proteins. Although both Tax proteins are highly homologous, Tax-1 does exhibit distinct structural features in which Tax-2 lacks. Tax-1 contains a PDZ binding motif (PBM) in its carboxyl-terminus that is important for binding to DLG-1 and other PDZ containing proteins, and these interactions were thought to play an important role for cell transformation by Tax-1. In addition, Tax-1, but not Tax-2, undergoes K63-linked polyubiquitination as part of its mechanism to activate NF-κB (Shembedze et al., 2007, Jounro et al., 2013). It is apparent that these differences do not account for the stronger transforming capability of Tax-2 in primary CD4+ T cells. Tax-1 may acquire a full transforming ability in human CD4+ T cells in cooperation with HBZ, an HTLV-1 antisense gene product that is constitutively transcribed and remains intact in ATL cells. In fact, HBZ itself was proven leukemogenic in mouse model. 30% of HBZ-transgenic mice developed spontaneous inflammatory lesions (Satou and Matsumura, 2012). Furthermore, increasing evidences showed that the functional interaction between Tax and HBZ is crucial for HTLV-1 oncogenesis in patients. Since high levels of Tax-1 expression lead to hyper-activation of NF-κB, consequently resulting in replicative senescence or even cell death (Zhu et al., 2011), HBZ antagonizes Tax-1’s toxic function to facilitate immune evasion and to promote cell cycle progression and proliferation. Interestingly, Tax-2 also induces hyper-activation of NF-κB in CD4+ T cells, and it is capable of promoting T cell proliferation in the absence of the antisense gene product encoded from HTLV-2. This suggests that unlike Tax-1, Tax-2 is a singly important viral component of the HTLV-2 genome to execute its transforming activity in CD4+ T cells.

We also found that the Tax proteins had their own preference on donor selection, and Tax-1 and Tax-2 immortalized CD4+ T cells from different blood donors. In one unusual case, Tax-1 immortalized CD4+ T cells while Tax-2 immortalized CD4+ myeloid dendritic cells (mDCs) from CD4+ cell pools of the same blood donor (Ren et al., unpublished data). These mDCs showed negative lineage markers (CD3−/CD4−/CD8−/TCRβ−/CD19−/CD56−/CD16−/CD14−/CD44+) and positive myeloid/dendritic cell markers (CD11c+CD123+/HLA-DR+CD32+CD74+CD117+/CD313+). Because the number of mDCs in healthy donors is typically less than 1%, the finding that Tax-2 is able to immortalize mDCs from enriched CD4+ pools suggests that the Tax-2-mediated immortalization of blood cells is highly selective. Aside from this finding, genotyping analysis of one Tax-2-established CD4 T cell line showed that these cells were clonal population. Together, our data demonstrate that in the absence of other viral components, Tax-2 alone is sufficient to promote clonal expansion of selected subsets of human primary CD4+ T cells. The potential cellular factor that contributes to Tax’s selectivity is currently unclear, which will certainly be an interesting subject for further investigation.

It is known that HTLV-1 has preferential tropism for CD4+ T cells in healthy carriers, HAM/TSP and ATL patients. In addition to utilization of GLUT1 as an entry factor for HTLV viruses, both viruses also utilize NRP1. HTLV-1 and -2 have a differential requirement for heparan sulfate proteoglycans (HSPG). Distinct from HTLV-1, HTLV-2 is not dependent on HSPGs for cell entry (Jones et al., 2006). Because HTLV-1 utilizes a ubiquitously expressed receptor for viral entry, it is expected that this virus would have the capacity to infect cell types other than CD4+ T cells. Indeed, CD8+ lymphocytes, monocytes and B-lymphocytes are found to harbor HTLV-1 (Koyanagi et al., 1993; Eiraku et al., 1998; Nagai et al., 2001). In addition, macrophages, dendritic cells, megakaryocytes as well as glial cells (astrocytes and microglial cells) are also the cell targets for HTLV-1 infection in vivo (Macatonia et al., 1992, Koyanagi et al., 1993; Levin et al., 1997; Grant et al., 2002). HTLV-1 isolates can be transmitted to primary human endothelial cells and basal mammary epithelial cells in vitro (Ho et al., 1984; Hoxie et al., 1984). Regardless of a broad cell tropism of HTLV-1, this virus exclusively causes malignant transformation of infected CD4+ T cells, suggesting that a panel of CD4 cell-specific cellular factors is crucial in assisting HTLV-1-mediated oncogenesis. Conversely, it is also possible that other types of cells may express cellular repressors to restrict HTLV-1 oncogenesis as seen in other retroviruses. For instance, SAMHD1 is able to restrict HTLV-1 replication in myeloid cells (St. Gelais and Wu, 2011). Unlike HTLV-1, HTLV-2 preferentially infects CD8+ T cells in human. However, Tax-2, when expressed by a pseudotype form of lentivirus, executes its transforming activity in CD4+ T cells. This phenomenon of disintegrated cell tropism (in CD8+ T cells) and disease-causing capacity (in CD4+ T cells) may partially explain why HTLV-2 does not currently cause leukemia in human.

These findings may have important implications on pathogenic virus evolution. Many viruses are considered non-pathogenic, because they do not apparently cause human disease based on current data. These non-pathogenic viruses usually do not receive much attention as known pathogenic viruses do. From an evolutionary point of view, recombination and cell tropism switch among homologous pathogenic and non-pathogenic viruses may occur, potentially generating a more lethal virus. A recent study...
demonstrated that cell tropism switch could be initiated by artificially fusing chimeric envelope proteins. Switch of the envelope SU domains between HTLV-I and HTLV-II alters the tropism of HTLV-II from CD4 to CD8 + T cells (Kaminari et al., 2013). In the case of HTLV-II, the infection rate among IV drug abusers is as high as 30%. Since no treatment is available for HTLV-II infection and host immunity is insufficient to eradicate this virus, it is possible that the rate of infection is going to rise due to the presence of accumulative HTLV-II reservoirs in host CD8 + T cells. Indeed, co-infection of HTLV-II with HTLV-I or with HIV-I has been documented and is increasing (Uchiyama et al., 1977; Shimoyama, 1991). Probably because these viruses share similar transmission pathways and risk factors. HTLV-II could be eventually modified in dendritic cells or monocytes that are co-infected with HTLV-I or HIV-I to acquire new cell tropism for CD8 + T cells, leading to its transformation of CD8 + T cells. Although naturally occurring cell tropism switch among homologous viruses may take unpredictable amount of time, this event does occur evolutionarily.

Although Tax-2 does not transform normal CD8 T cells, this viral protein exhibits its transforming activity in CD8 + T cells derived from T cell type of large granular lymphocyte leukemia (T-LGLL). T-LGLL leukemia is the malignancy of CD8 + cytotoxic T cells, which usually occurs in elderly patients. The disease course is frequently associated with autoimmune diseases (Rose and Berliner, 2004). Primary T-LGLL leukemia cells display the CD3 +CD8 +CD57 + SU markers, representing activated cytotoxic T lymphocytes. Despite T-LGLL cells are leukemia cells, these cells do not grow in culture. By expressing Tax-2 in these cells, long-term growth and clonal expansion of T-LGLL cells could be achieved (Ren et al., unpublished data).

In summary (see Table 1), Tax-1 and Tax-2 display differential transforming activities in human T lymphocytes. Tax-2 demonstrates a more potent activity than Tax-1 in immortalizing human terminally differentiated CD4 + T cells. Besides, Tax-2, not Tax-1, is able to transform CD8 + T cells from T-LGLL leukemia cells with preexisting oncogenic profile. The ability of Tax-2 to establish specific subsets of T cell lines implicates its utilization in studying human T cell biology and in developing leukemia model. Furthermore, the hypothesis that Tax-2 has a disease-causing capacity in CD4 + T cells needs to be validated in humanized mouse model.

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Table 1 | Differential activities of Tax-1 and Tax-2 in human T cells.

| Tax-1 | Tax-2 |
|---|---|
| Activation of | + | + |
| Rex/ANF+4B | + | + |
| Stat3 | + | + |
| PKC/Akt | + | + |
| AP-1 | + | + |
| CREB | + | + |
| Dysregulation of autophagy | + | - |
| Lipid raft involvement | + | - |
| Immortalization of | CD4 + T cells | + | + |
| CD8 + T cells | - | - |
| CD8 + T cells from T-LGLL | - | - |

*Tax-1 recruits kinesin into lipid raft microdomains for persistent activation of NF-κB signaling, while Tax-2 activation of NF-κB does not appear to involve lipid raft pathways (Fregni et al., 2008). Tax-1 is able to immortalize human primary CD4 + T cells with low efficiency (2 out of 12), and Tax-1-immortalized T cells grow slowly in culture and experience spontaneous cell death. Tax-2 immortalizes human primary CD4 + T cells more efficiently than Tax-1 (4 out of 12). Tax-2-immortalized T cells grow in culture at a rate comparable to some lymphoblastic leukemia cell lines with healthy growth appearance.*
human endوهedral cells by human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. U.S.A. 81, 2791–2795.

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