T memory stem cells are the hierarchical apex of adult T-cell leukemia

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Abstract

Background & Aims

Adult T-cell leukemia (ATL) is a mature $CD4^+$ T cell neoplasm associated with human T-cell leukemia virus type 1 (HTLV-1).[1,2] Despite a number of investigations using human specimens and mice models, the exact origin of ATL cells remains unclear.[3-7] Recently T memory stem (T_{SCM}) cells were identified as a unique $CD45RA^+$ T cell subpopulation with stem cell-like properties such as enhanced self-renewal capacity and multipotency.[8] In this study, we investigate the phenotypic and functional heterogeneity and the hierarchy of ATL cells and whether T_{SCM} cells are involved in the development of ATL.

Materials & methods

Clinical samples from healthy individuals (HIs), HTLV-1 carriers, and ATL patients were collected after written informed consent was obtained. To separate $CD4^+$ T cells into four subpopulations including naïve T (T_N), T_{SCM}, central memory T (T_{CM}), and effector memory T (T_{EM}) cells, we adapted 8 colors FACS sorting according to a previously described protocol[8,9] with minor modifications. Peripheral blood mononuclear cells (PBMCs) were stained with anti-CD45RO, anti-CCR7, anti-CD95, anti-CD122, anti-CD25, anti-CD4, and anti-CD45RA, in addition to a cocktail including anti-CD11b, anti-CD14, and anti-CD56. Qualitative and

quantitative PCR were performed for the pX region which is relatively conserved in the HTLV-1 genome, and RNase P as the internal control. HTLV-1 proviral load (PVL) was described as the relative value of pX/RNase P to that in $CD3^+CD4^+CD25^+$ cells from an ATL patient with single provirus integration proven by Southern blot hybridization analysis. Inverse long PCR was performed to identify ATL clones among numerous HTLV-1-infected cells as previously described.[10] ATL clone-specific integration sites were determined by direct sequencing of inverse long PCR products, and ATL clone-specific PCR assay was constructed in the individual cases. Each sorted $CD4^+$ T cell subpopulation was cultured in Iscove's modified Dulbecco's medium supplemented with recombinant human interleukin 7 (IL-7) for 2 weeks and analyzed for the phenotypic change. NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice,[11] NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic (NOG) mice,[12] and NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ (NRG) mice[13] were used for xenogeneic transplantation assay and repopulating human cells were analyzed for phenotype and clonality.

Results

We first investigated whether hematopoietic stem and progenitor cells (HSPCs) were infected with HTLV-1. We obtained bone marrow mononuclear cells from five ATL patients and sorted $lineage^-CD34^+$ cells that contain HSPCs but not mature hematopoietic cells including T, B, natural killer, myeloid, and erythroid cells. PCR which can successfully amplify five or greater copies of the pX region showed that no amplification was observed in HSPCs from all tested samples, indicating that HTLV-1-infected cells do not exist in HSPCs.

$CD4^+$ T cells from PBMCs of HIs were successfully separated into four subpopulations including T_N ($CD4^+CCR7^+CD45RA^+CD45RO^-CD95^-CD122^-$), T_{SCM} ($CD4^+CCR7^+CD45RA^+CD45RO^-CD95^+CD122^{dim}$), T_{CM} ($CD4^+CCR7^+CD45RA^-CD45RO^+$), and T_{EM} ($CD4^+CCR7^-CD45RA^-CD45RO^+$) in agreement with previous studies.[14] We applied the same gating method to PBMCs from 3 HTLV-1 carriers and 17 ATL patients and found

that all four populations were separable across all disease phases. Numbers of entire $CD45RO^+$ memory T cells (T_{CM} plus T_{EM}) were increased in ATL patients consistently with the clinical aspect that the majority of ATL cells express CD45RO, whereas numbers of T_{SCM} cells were mostly comparable between HIs and ATL patients. To examine the exact distribution of HTLV-1-infected cells in $CD4^+$ T cell subpopulations, we sorted these four subpopulations and measured PVL. The majority of T_{CM} and T_{EM} cells were infected with HTLV-1 in agreement with a previous study reporting the correlation of $CD4^+$ memory T cell number and PVL.[15] Intriguingly, PVL of T_{SCM} cells was clearly measurable in all tested cases including not only ATL patients but also HTLV-1 carriers, whereas PVL of T_N cells was barely detectable in only half of tested cases at the level which is explicable as contamination. Furthermore to investigate the distribution of ATL clones in $CD4^+$ T cell subpopulations, we constructed ATL clone-specific PCR that amplified the unique flanking region of HTLV-1 integration site in the individual cases. In all tested cases, each targeted site was amplified not only in T_{CM} and T_{EM} cells but in T_{SCM} cells, while no amplification was detected in T_N cells. These results indicate that ATL clones are composed of phenotypically heterogeneous memory T cells including T_{SCM}, T_{CM}, and T_{EM} cells.

Next, to investigate the possibility of functional hierarchy among T_{SCM}, T_{CM}, and T_{EM} cells, we purified each memory T cell population and performed an in vitro culture assay with IL-7 that promotes survival but not differentiation of $CD4^+$ T cells.[16] Interestingly T_{SCM} cells from HTLV-1 carriers and ATL patients sustained the original phenotype and concurrently generate $CD45RO^+$ memory T cells, whereas the opposite induction from T_{CM} or T_{EM} cells to T_{SCM} cells was never observed in all tested cases. These results indicate that T_{SCM} cells from HTLV-1 carriers and ATL patients are hierarchically upstream of $CD45RO^+$ memory T cells.

Finally to investigate tumorigenicity of T_{SCM} cells, we performed a xenogeneic transplantation assay. We inoculated 2 to 3×10^4 cells of sorted T_N, T_{SCM}, T_{CM}, or T_{EM} cells from one ATL

case intraperitoneally into immunodeficient mice and evaluated the phenotype and clonal architecture of repopulating cells in mice. Unidirectional differentiation from T_{SCM} to T_{CM} and T_{EM} was reconfirmed and T_{SCM} cells displayed high repopulating capacity of the identical ATL clone compared to other $CD45RO^{+}$ memory T cells. These results indicate that T_{SCM} cells are competent to preserve ATL clones and to give rise to ATL cells.

Conclusions

In this study, we clearly demonstrated the presence of ATL clones in T_{SCM} population as well as in T_{CM} and T_{EM} populations. Our in vitro culture and xenogeneic transplantation assay showed unidirectional differentiation from T_{SCM} to T_{CM} and T_{EM} and that T_{SCM} cells have high repopulating capacity of the identical ATL clone. Collectively, ATL cells are phenotypically and functionally heterogeneous. T_{SCM} cells are identified as the hierarchical apex capable of reconstituting the identical ATL clones and could be the source for development of ATL.

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