Molecular characterization of heterogeneity in Adult T-cell leukaemia/lymphoma

Huseini Kagdi, 1 Aileen Rowan, 1 Maria Antonietta Demontis, 1 Charles Bangham, 1 Graham Taylor 1

1 Division of Infectious Disease, Imperial College London, London, United Kingdom

INTRODUCTION

HTLV-1 is a complex delta retrovirus infecting ~10 million people worldwide. ~4-7% infected carrier (AC) develop Adult T cell Leukaemia/lymphoma (ATL), a mature T cell neoplasm and similar number develop HTLV-1 associated myelopathy (HAM). ATL has highly heterogeneous clinical features and prognosis. Shimoyama et al proposed classification into 4 subtypes: smouldering, chronic, acute and lymphoma; the former two being indolent (Ind) while latter aggressive (Agg) ATL respectively (1). Long term longitudinal studies suggest majority of Ind ATL progress to Agg ATL (2). Non ATL HTLV-1 infected individual (AC and HAM; HTLV+) with high Proviral Load (PVL) [HTLV+hi] have features overlapping with Ind ATL and are at higher risk of progression to ATL as shown in figure 1(3). The precise molecules features underlying the malignant progression though HTLV+hi, Ind and Agg ATL remains unknown. Hence it is impossible to predict, treat and prevent ATL. We aim to characterize the immunophenotype of HTLV-1 infected cells in patient with HTLV+hi, Ind ATL and Agg ATL. We hypothesise the immunophenotype of HTLV-1 infected cells in non ATL HTLV-1 infection (AC and HAM) with or without high PVL, indolent ATL with or without progressive disease and aggressive ATL is different and can help characterize the malignant progression in HTLV infection and ATL.

MATERIAL AND METHOD

58 patients with HTLV-1 infection attending the National Centre for Human Retrovirology, St Mary’s hospital, London as characterized in Table 1 provided blood samples after informed written consent. Samples from 10 uninfected individuals and 2 HTLV-1 immortalized cell lines (MT2, C8 166) were used as controls. Peripheral blood mononuclear cells (PBMCs) were isolated by specific gravity mediated centrifugation and stored in 10% DMSO at -160º C. PBMCs were then thawed and stained by fluorochrome conjugated antihuman antibodies (CD3, CD4, CD7, CD8, CD25, CD26, CD127, CCR4 and CCR7). The samples were interrogated on BD LSR II/ Fortessa and analysed using flowjo© software. PVL was estimated using real time PCR and clonality using Ligation mediated PCR. Statistical analysis (non parametric t test / ANOVA/spearman) and liner regression was performed using GraphPad prism© software.

RESULTS

The frequency of CD4+CCR4+ T-cells, was significantly increased in HTLV+hi individuals with PVL >5% [HTLV+hi] & ATL compared to uninfected individuals & HTLV-1+ with PVL <5% [HTLV-] and correlated highly significantly with PVL as shown in figure 2. PVL within CCR4+ T-cells contributed to 93% to total T cells PVL suggesting CD4+CCR4+ T-cells are the main reservoir of HTLV-1 infection. CD26- population within CD4+CCR4+ T-cells contributed to 22% of infected burden and 32% of ATL were CD26- suggesting CD26 up-regulation is non specific for malignant progression. CD26 expression within CD4+CCR4+ T-cells showed stepwise reduction in HTLV+hi & ATL and correlated with distribution of PVL as shown in Figure 2. This suggests CD26 down-regulation as specific premalignant change. CD7 expression within CD4+CCR4+CD26- T-cells in ATL was significantly lower. CCR7 expression within CD4+CCR4+CD26-CD7- was significantly higher in aggressive ATL and CD127 expression with CD4+CCR4+CD26-CD7- was higher in stable indolent ATL as shown in figure 3.

CONCLUSION

CD4+CCR4+ T-cells are the main reservoir of HTLV-1 infected T-cells. CD26 down-regulation is an early malignant change while CD7 down-regulation identifies clinical ATL. CCR7 up-regulation and/or CD127 is associated with aggressive ATL. CCR7+ ATL is associate within refractory disease. These finding help diagnose and prognosticate ATL. Isolation of T cell subset based on these expression provides opportunity to further characterize ATL leukemogenesis.

REFERENCES


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