Kinetic analysis of Human T-cell Leukemia Virus type 2 expression in chronically-infected cells and patient PBMCs

Cecilia Bendera, Paola Righib, Francesca Rendeb, Paola Ronzic, Claudio Casoli, Vincenzo Ciminale and Umberto Bertazzoni

a) Dept of Mother and Child, Biology and Genetics, Università degli Studi di Verona; b) Dept. of Oncology, Università degli Studi di Padova; c) Dept of Clinical Sciences, Università degli Studi di Milano, Italy

INTRODUCTION
The elucidation of the viral gene expression profile provides useful information in assessing the function of specific viral genes in the process of infection and cellular transformation. HTLV-2 pattern of mRNAs expression produces three major classes of mRNAs: unspliced genomic mRNA for Gag, protease and Pol proteins; singly spliced mRNAs encoding Env and the accessory proteins p28, p22/p20-1 and -2; and a doubly spliced mRNA for the regulatory proteins Tax, Rex and for the p10/p11 and p7 accessory ones (Ref.1 and Fig. 1). To date, very little information has been obtained on the temporal regulation of different HTLV-2 transcripts expression in infected cells. Aim of this study was to investigate the kinetics of gene expression from HTLV-2 infected cell lines and from PBMCs of HTLV-2B infected subjects. The expression profile and kinetics of the different transcripts were analysed by real time RT-PCR using splice-junction-specific primers.

METHODS
The levels of different transcripts expression was evaluated in different HTLV-2 chronically infected cell lines in log phase of growth. The levels of expression of three major classes of HTLV-2 mRNAs were measured at different time points in BJAB-Gu infected human B-cell line and in IL-2 stimulated PBMCs from HTLV-2B infected patients. In order to synchronise viral expression, BJAB-Gu cells were plated at 1/10 of optimally growing concentration and analysed every 24 hours. Cryopreserved PBMCs were plated and analysed at closer time points. Total RNA was subjected to reverse transcription, resulting cDNAs were amplified by real time quantitative PCR using different combinations of splice-junction-specific primers pair and probes (Fig. 2). Transcripts copy number were normalised to that obtained for the 18sRNA.

RESULTS
This approach was used to first determine the steady-state levels of expression for the different viral transcripts in three different cell lines in log phase of growth (Fig. 3). Experiments performed indicated that gag/pol is the most abundant transcript. The expression level of env was comparable in the two T-cell lines, Mo-T and C344, infected by the 2A subtype, and was considerably higher than in the B-cells infected with HTLV-2B subtype, where p10/p11 and p7 transcripts were below the limit of detection. The elucidation of the viral gene expression follows a distinct timing both in infected cell lines and in PBMCs of HTLV-2B infected subjects. The transcript env is the first to be expressed, this indicating that it is necessary at the beginning of the infection cycle to transactivate and regulate viral and cellular transcripts. These results also suggest that the control of viral gene expression is highly regulated both in its kinetics and expression level.

CONCLUSIONS
The expression of different HTLV-2 genes follows a distinct timing both in infected cell lines and PBMCs isolated from infected patients. The transcripts tax/rex is the first to be expressed, thus indicating that is necessary at the beginning of the infection cycle to transactivate and regulate viral and cellular transcripts. These results also suggest that the control of viral gene expression is highly regulated both in its kinetics and expression level.

REFERENCES