UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 AND CHEMOSENSITIVITY OF LEUKAEMIA AND LYMPHOMA CELLS IN ADULT PATIENTS IN MALAYSIA

VIKNESVARAN SELVARAJAN

FPSK(M) 2007 10
PREVALENCE OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 AND CHEMOSENSITIVITY OF LEUKAEMIA AND LYMPHOMA CELLS IN ADULT PATIENTS IN MALAYSIA

By

VIKNESVARAN SELVARAJAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

August 2007
To my parents ...  
mentors and friends
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PREVALENCE OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 AND CHEMOSENSITIVITY OF LEUKAEMIA AND LYMPHOMA CELLS IN ADULT PATIENTS IN MALAYSIA

By

VIKNESVARAN SELVARAJAN

August 2007

Chairman: Zamberi Sekawi, PhD

Faculty: Medicine and Health Sciences

The elucidation of virus-cancer associations is of particular importance since large numbers of people are potentially exposed to cancer. The first link relates to the causation of adult T-cell leukaemia/lymphoma (ATL), a highly malignant haematological malignancy of mature activated T cells with a poor prognosis, by a retrovirus called human T-cell lymphotrophic virus type-1 (HTLV-1). The HTLV-1 tax oncoprotein plays an integral role in productive viral replication and disease progression. Seroprevalence studies demonstrated that the distribution of HTLV-1 is heterogeneous worldwide and not specific to a particular region only. Patients with this disease have a very poor prognosis because of intrinsic chemoresistance and severe immunosuppression. Hence, the general objective of the present study is to establish the prevalence of HTLV infections in leukaemia and lymphoma adult patients. The experimental design consists of two folds: screening for the presence of HTLV-1 tax gene and chemosensitivity profiles of patient cells treated with clinical chemotherapeutic agents. A total of 140 subjects
consisted of lymphoid leukaemia (12%), myeloid leukaemia (26%) and lymphoma patients (62%) were included in this study. First line screening was performed using ELISA and PCR was used to detect HTLV-1 tax gene followed by confirmation using direct DNA sequencing. Mononuclear cells were isolated using density gradient centrifugation from bone marrow or peripheral blood samples of adult patients admitted to Universiti Malaya Medical Centre (UMMC), Ward 6TD. Patient cells were treated based on standard chemotherapeutic regimen for 96 hours and assessed using 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. Initial ELISA screening showed 9 samples were initially reactive and 7 patients were classified indeterminate due to inconsistency of immunoassay replicates. Further confirmation by PCR validated all seropositive patients and only four of the indeterminate samples, which yields a prevalence of 9.29% in 140 adult patients. Concurrently, the HTLV-1 tax positive patient’s chemosensitivity profiles were compared with the seronegative samples. However, a distinct relationship between the presence of HTLV-1 tax gene and chemosensitivity between these groups were not obtained. This preliminary study provided a baseline data on the prevalence of HTLV-1 infections in leukaemia and lymphoma adult patients. However, the lack of direct association of HTLV-1 tax gene with the chemotherapy resistance was mainly due to the limited sample size used in this study. Further studies should be performed in a larger cohort of patients and healthy subjects to further substantiate the preliminary data.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PREVALENS HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 DAN CHEMOSENSITIVITI SEL-SEL PESAKIT DEWASA LEUKEMIA DAN LIMFOMA DI MALAYSIA

Oleh

VIKNESVARAN SELVARAJAN

Ogos 2007

Pengerusi: Zamberi Sekawi, PhD

Fakulti: Perubatan dan Sains Kesihatan

penyaringan gen tax HTLV-1 dan profil kemosensitiviti sel pesakit selepas dirawat menggunakan agen-agen kemoterapeutik. Sejumlah 140 sampel merangkumi limfoid leukaemia (12%), myeloid leukaemia (26%) dan juga kes-kes limfoma (62%) telah diambil kira. Penyaringan dasar adalah menggunakan ELISA dan PCR digunakan untuk mengesan gen tax HTLV-1 serta diikuti oleh DNA sekuensing sebagai kaedah pengesanan. Sel-sel mononuklear daripada sampel darah dan sum-sum tulang pesakit dewasa yang menerima rawatan di Hospital Universiti Malaya (UMMC), Wad 6TD diasingkan melalui centrifugasi kecerunan tumpat. Sel-sel pesakit dirawat melalui regimen umum untuk 96 jam menggunakan kaedah 3-(4, 5-dimetiltiazol-2)-2, 5-difeniltetrazolium bromida (MTT) sitotoksik. Penyaringan dasar menggunakan teknik ELISA menunjukkan 9 sampel adalah reaktif dan 7 sampel dikategorikan sebagai ketidakpastian kerana ulangan kaedah yang tidak konsisten. PCR menunjukkan semua seropositif pesakit dan hanya empat daripada sampel-sampel yang tidak tentu adalah positif untuk HTLV-1 dengan prevalens 9.29% dalam 140 pesakit dewasa. Dalam pada itu, profil kemosensitiviti pesakit-pesakit HTLV-1 positif diasas dengan sample-sampel seronegatif. Hakikatnya, tida suatu hubungkait yang kukuh di antara dua kumpulan pesakit. Kajian awal ini telah menunjukkan data dasar mengenai prevalens jangkitan HTLV-1 di kalangan pesakit-pesakit leukemia dan limfoma dewasa. Hubungkaitan yang kukuh dengan gen tax HTLV-1 dan kesan kemoterapeutik adalah kurang jelas kerana saiz sampel yang sangat kecil. Kajian lanjut perlu dijalankan di dalam kohort sampel yang lebih besar dari golongan pesakit serta subjek-subjek sihat untuk menegaskan data awal dari kajian ini.
Although I am indeed the sole author of this thesis, I am by no means the sole contributor! So many people have contributed to my thesis, to my education, and to my life, and it is now my great pleasure to take this opportunity to thank them.

First and foremost, I am deeply indebted to my respectable supervisor, Dr Zamberi Sekawi, who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my Masters degree to his encouragement and effort. One simply could not wish for a better or friendlier supervisor.

I would like to express my sincere and heart-felt gratitude to my co-supervisor, Dr. Johnson Stanslas who has stood by me along the course of this research project. If not for him, I would not have proposed this research, what more carry it out successfully. I also thank him for motivating and for guiding me.

I would also like to take this opportunity to thank Dr. Vijay Sangkar primarily for accepting my proposal and to have supervised my sample collection. Sincere gratitude to Dr. Shankar in putting up the proposal of my research project to the UMMC Ethics Sub-Committee. Special thanks to Dr. Muthukumaran, the person responsible for taking me into UMMC to proceed with my research, and introducing me to Dr. Shankar and Dr. Vijay Sangkar.
I must surely thank Pn. Rahani (Administrative clerk of UMMC Ward 6TD) for securing the patient samples till collection, the oncology nurses and the clinical oncologist at UMMC for their support and help.

I would like to express my appreciation to Assoc. Prof. Dr. Rozita Rosli, Mr. Nasir, Dr. Thilagawathy, Assoc. Prof. Dr. Mariana, Dr. Neela Kumari, Mr. Nagi, Ms. Wan, and Ms. Farah for allowing me to learn some essential techniques for my research at their laboratory. Special thanks to all the staff of FMHS, UPM, especially Mr. Tung Chee Keong, Mr. Rizal, Ms. Suzanna, Pn. Safarina, Pn. Fadzrina, Mr. Zainan, Mr. Anthonsamy and Mr. Ayahsamy for their help.

I am grateful to my labmates, Lim Siang Hui, Tang Seng Chuan, Audrey, Guru, Riyadh, Jebril, Dr. Noor Wijayahadi and others for their help and sharing wonderful moments being together at the CRDD group.

I would also like to extend my gratitude to my friends and all my loved ones especially Velan, Sagineedu and Dr. Srinivas for their immeasurable support and help, generously provided insightful ideas and suggestions and by just being together throughout the study.

Finally, I would like to acknowledge the loving support of my family during the writing of this thesis. Their fortitude, inspiration and enduring patience helped me make the job an enjoyable adventure.
I certify that an Examination Committee has met on 14 August 2007 to conduct the final examination of Viknesvaran Selvarajan on his Master of Science thesis entitled "Prevalence of Human T-Cell Lymphotropic Virus Type 1 and Chemosensitivity of Leukaemia and Lymphoma Cells in Adult Patients in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Farida Jamal, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Eusni Rahayu Mohd. Tohit, PhD
Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Latifah Saiful Yazan, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Ilina Isahak, PhD
Professor
Faculty of Medicine
Universiti Kebangsaan Malaysia
(External Examiner)

___________________________
HASANAH MOHD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Zambri Sekawi, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Johnson Stanslas, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

______________________  
**AINI IDERIS, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date: 10 July 2008
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at UPM or at any other institutions.

__________________________
VIKNESVARAN SELVARAJAN

Date: 10 July 2008
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xvi</td>
</tr>
</tbody>
</table>

CHAPTER

1 INTRODUCTION
1.1 Background 18
1.2 Research Significance 20
1.3 Objectives
  1.3.1 General Objective 20
  1.3.2 Specific Objectives 20

2 LITERATURE REVIEW
2.1 Introduction 21
2.2 Discovery of HTLV 22
2.3 Genomic Structure 23
2.4 Epidemiology 26
2.5 Transmission 29
2.6 Methods of Detection 30
2.7 Disease Association 32
  2.7.1 Adult T-cell Leukaemia/Lymphoma 32
  2.7.2 HTLV-1 associated Myelopathy/Tropical Spastic Paraparesis 35
2.8 Mechanism of Infection 36
2.9 Prevention 37
2.10 Leukaemia 38
2.11 Risk Factors 39
2.12 Acute Lymphoblastic Leukaemia 40
  2.12.1 Overview 40
  2.12.2 Clinical Features 41
  2.12.3 Treatment 42
2.13 Acute Myeloid Leukaemia 42
  2.13.1 Overview 42
  2.13.2 Clinical Features 43
  2.13.3 Treatment 44
2.14 Lymphoma 45
2.15 Non-Hodgkin’s Lymphoma 46
3 MATERIALS AND METHODS
3.1 Patient Samples 54
3.2 Mononuclear Cells Isolation and Culture 55
3.3 Cell Proliferation
   3.3.1 Chemotherapeutic Drugs 57
   3.3.2 Drug Cytotoxicity Assay 57
3.4 ELISA 59
3.5 Genomic Extraction 60
3.6 Detection of Tax Gene 62
3.7 PCR Product Purification 64
3.8 Sequencing of Tax Gene 65
3.9 Statistical Analysis 67

4 RESULTS AND DISCUSSION
4.1 Patients 68
4.2 Serological Analysis 69
4.3 Molecular Analysis 70
4.4 Cytotoxicity Profiles 72
4.5 Discussion
   4.5.1 Application of MTT Assay 77
   4.5.2 Drug Resistance 78
   4.5.3 Role of Tax in Drug Resistance 85

5 CONCLUSIONS 88

REFERENCES 90
APPENDICES 106
BIODATA OF CANDIDATE 116
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Tables</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Products of HTLV-1 genes</td>
</tr>
<tr>
<td>2</td>
<td>FAB classification system for AML</td>
</tr>
<tr>
<td>3</td>
<td>Clinical presentation of lymphomas</td>
</tr>
<tr>
<td>4</td>
<td>Concentration ranges of the drugs tested</td>
</tr>
<tr>
<td>5</td>
<td>Primer pairs of tax sequence</td>
</tr>
<tr>
<td>6</td>
<td>Seropositive patients by disease association</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Genomic structure of HTLV-1</td>
</tr>
<tr>
<td>2</td>
<td>Worldwide prevalence of HTLV-1</td>
</tr>
<tr>
<td>3</td>
<td>Typical leukaemic cells with multilobulated nuclei ‘flower cells’ in the peripheral blood of an acute ATL patient</td>
</tr>
<tr>
<td>4</td>
<td>Major factors influencing aetiology of cancer</td>
</tr>
<tr>
<td>5</td>
<td>Centrifugation of samples using Histopaque®-1077</td>
</tr>
<tr>
<td>6</td>
<td>Schematics of a typical microculture plate</td>
</tr>
<tr>
<td>7</td>
<td>Procedures for HTLV-I/II ELISA 3.0</td>
</tr>
<tr>
<td>8</td>
<td>Disease Entities</td>
</tr>
<tr>
<td>9</td>
<td>Demographic Profiles based on Ethnicity, Sex and Age</td>
</tr>
<tr>
<td>10</td>
<td>Detection of tax gene in seropositive patient samples</td>
</tr>
<tr>
<td>11</td>
<td>Detection of tax gene in indeterminate patient samples</td>
</tr>
<tr>
<td>12</td>
<td>Illustration of a Dose-Response curve</td>
</tr>
<tr>
<td>13</td>
<td>Chemosensitivity Profiles of ALL Patients</td>
</tr>
<tr>
<td>14</td>
<td>Chemosensitivity Profiles of AML Patients</td>
</tr>
<tr>
<td>15</td>
<td>Chemosensitivity Profiles of Lymphoma Patients (A)</td>
</tr>
<tr>
<td>16</td>
<td>Chemosensitivity Profiles of Lymphoma Patients (B)</td>
</tr>
<tr>
<td>17</td>
<td>Chemosensitivity Profiles of Pooled Tax Positive Patients</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>Acute lymphoblastic leukaemia</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloid leukaemia</td>
</tr>
<tr>
<td>ATL</td>
<td>Adult T-cell leukaemia/lymphoma</td>
</tr>
<tr>
<td>BLV</td>
<td>Bovine leukaemia virus</td>
</tr>
<tr>
<td>BMT</td>
<td>Bone marrow transplantation</td>
</tr>
<tr>
<td>CLL</td>
<td>Chronic lymphocytic leukaemia</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic myeloid leukaemia</td>
</tr>
<tr>
<td>CREB</td>
<td>Cyclic AMP responsive element binding protein</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular coagulopathy</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assays</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>FAB</td>
<td>French-American-British</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Glucose transporter type 1</td>
</tr>
<tr>
<td>HAM/TSP</td>
<td>HTLV-1-associated myelopathy/ tropical spastic paraparesis</td>
</tr>
<tr>
<td>HL</td>
<td>Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>Human T-cell lymphotropic virus type-1</td>
</tr>
<tr>
<td>HTLV-2</td>
<td>Human T-cell lymphotropic virus type-2</td>
</tr>
<tr>
<td>IVDU</td>
<td>Intravenous drug users</td>
</tr>
<tr>
<td>LTRs</td>
<td>Long terminal repeats</td>
</tr>
<tr>
<td>MTOC</td>
<td>Microtubule organising centre</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>NHL</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
</tbody>
</table>
ORFs  Overlapping reading frames
PCR  Polymerase chain reaction
PTLVs  Primate T-cell leukaemia viruses
Rex  Regulatory gene of ‘X’ region
SRF  Serum-responsive factor
STLV  Simian T-leukaemia virus
Tax  Transactivating gene of ‘X’ region
WB  Western blot
WBC  White blood cell
CHAPTER 1
INTRODUCTION

1.1 Background

Since the dawn of time, transformation of normal cells of an animal into cancer cells is a process which can be induced experimentally by a variety of agents. A perplexing aspect of these experiments is the difficulty or impossibility, of formulating a unitary mechanism for the various agents. For instance, cancer can be induced equally well by substances which have a strong action on the nucleic acids, or by hormones, or even by completely inert substances, such as sheets of plastic inserted under the skin. The lack of similarity in the properties of cancer-inducing agents has suggested that the similarity lies in the cellular mechanisms that they affect.

In the past 25 years, revelations on the genesis of human cancer have come at an increasing pace. The contributions of knowledge about oncogenic infectious agents, especially viruses, have been instrumental in that understanding because in transforming cells they mirror, often brilliantly, basic cellular processes that culminate in cancer. Infectious agents, chiefly viruses, are accepted causes or candidates as causes of diverse malignancies of people worldwide. From a universal perspective infectious agents especially viruses account for several of the most common malignancies – up to 20% of all cancers (Pagano et al., 2004). Some of these cancers are endemic with high incidence in certain geographic locations, but have sporadic low incidence in other parts of the world. The consistency of association of a given virus and a specific malignancy ranges from
essentially 100% to as low as 15% depending on the virus, the cancer and the geographic location.

The elucidation of virus-cancer associations is of particular importance since large numbers of people are potentially exposed. The possible role of viruses in leukaemogenesis has recently drawn considerable attention, and some links between viruses and leukaemia appear to exist. The first link relates to the causation of adult T-cell leukaemia/lymphoma (ATL), a highly malignant haematological tumour with a poor prognosis, by a retrovirus called human T-cell lymphotropic virus type-1 (HTLV-1). This virus is endemic in Southern Japan, the Caribbean and parts of Central Africa with prevalence in the general population of up to 30% and more (Tajima & Cartier, 1995). The lifetime risk of ATL among infected persons has been estimated to be 3.0% for women and 6.9% for men, with a long latency period of 30 years or more (Kondo et al., 1989). An infection in early period of life is particularly dangerous, and preventive measures in endemic areas should focus on maternal-infant transmission dynamics. In certain geographic areas, about 60%–80% of ATL are being attributed to this retrovirus (Manns et al., 1993), which is not only transmitted via breastfeeding, but also through other routes. Among intravenous drug users, HTLV-seropositivity is a common finding (Briggs et al., 1995).
1.2 Research Significance

This research was set to be an initial step on the prevalence study of HTLV infections in this part of the region.

It was a preliminary study of these viruses that could later form a basis for further comprehensive research in the development of routine clinical diagnostics and treatment protocols for HTLV infections.

1.3 Objectives

1.3.1 General Objective

To establish the prevalence of HTLV infections in leukaemia and lymphoma patients.

1.3.2 Specific Objectives

1. To investigate the presence of HTLV in adult patients diagnosed with leukaemia or lymphoma.

2. To identify and characterise HTLV based on serology and genomic techniques.

3. To evaluate in vitro chemosensitivity profiles of HTLV-infected as well as non-infected leukaemia or lymphoma cells to standard chemotherapeutic drugs.
CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1), which is also known as Human T-cell leukaemia virus, belongs to the genus deltaretrovirus, which includes HTLV-2, bovine leukaemia virus (BLV) and simian T-leukaemia virus (STLV). As well as HTLV-1, the latter two viruses cause lymphoid malignancies in the host. Since STLV and HTLV share the same molecular, virological, and epidemiological features, they are designated as primate T-cell leukaemia viruses (PTLVs).

HTLV-1 is closely related to STLV-1, a virus isolated from old world monkeys in Africa. Phylogenetic analysis indicates that HTLV-1 originated in Africa, and it seems that interspecies transmission from nonhuman primates to man also occurred in Africa and disseminated to other locations by the migration of Africans to other continents around 27300 ± 8200 years ago (Van Dooren et al., 2001). Nevertheless, it does not explain HTLV-1 isolation from Ainu indigenous people in Japan and aborigines in Melanesia and Australia.

HTLV-1 subtypes include subtype A, also known as the cosmopolitan subtype, which includes the prototype HTLV-1 sequence from Japan (Seiki et al., 1982) and is found in many HTLV-1-endemic areas worldwide, subtypes B, D, and F from Central Africa, subtype E from South and Central Africa (Slattery et al., 1999) and subtype C from Melanesia (Gessain et al., 1991; Sherman et al., 1992). HTLV-1 sequences may also be compared to STLV-1
isolates from non-human primate species (Vandamme et al., 1994). For most HTLV-1 subtypes, STLV-1 isolates from the same geographic region cluster closely with the human HTLV-1, suggesting multiple episodes of simian to human transmission and a probable African origin of HTLV-1 (Koralnik et al., 1994; Vandamme et al., 1994). Close sequence similarity between HTLV-1c and STLV-1 isolated from Macaca arctoides in Melanesia is an example of this phenomenon (Mahieux et al., 1997).

2.2 Discovery of HTLV

HTLV-1 was first identified in T-cell lymphoblastoid cell lines and fresh peripheral blood lymphocytes obtained from a patient with cutaneous T-cell lymphoma (mycosis fungoides) (Poiesz et al., 1980). This virus was associated with adult T-cell leukaemia/lymphoma (ATL) (Uchiyama et al., 1977) because it was observed that the cell line established from peripheral blood lymphocytes of a patient with ATL, produced antigens that reacted against sera from ATL patients (Hinuma et al., 1981). It was also associated with HTLV-1-associated myelopathy/ tropical spastic paraparesis (HAM/TSP) due to the prevalence of the antibody against this virus in the serum from TSP patients (Gessain et al., 1985) and, in the serum and cerebrospinal fluid from HAM patients (Osame et al., 1986).

Conversely, HTLV-2 was first identified in a cell line established from the spleen of a patient with hairy-cell leukaemia (Kalyanaraman et al., 1982). This virus has not yet been associated with any specific disease, although some HTLV-2 infected patients have been reported to be affected by atypical
T-cell hairy-cell leukaemia of large granular lymphocyte leukaemia (Rosenblatt et al., 1986), and tropical ataxic neuropathy (Sheremata et al., 1993).

2.3 Genomic Structure

HTLV-1 virions are complex type C particles, spherical, enveloped and 100–110 nm in diameter. The viral genome consists of a linear, positive sense, ssRNA held together by hydrogen bonds. Each monomer has about 9032 nucleotides. The 3’ terminal viral genome is polyadenylated and its 5’-terminal is capped. Each unit is associated with a specific molecule of tRNA that is base paired to a region, primer binding site, near the 5’ end of the RNA. Proviral forms are flanked at both termini by long terminal repeats (LTRs) of 754 nucleotides.

The genomic structure encodes structural and enzymatic proteins: gag, pol, env, reverse transcriptase, protease, and integrase (Franchini, 1995). In addition, HTLV-1 has a region at the 3’ end of the virus, called pX, which encodes four partially overlapping reading frames (ORFs) (Figure 1). These ORFs code for regulatory proteins which impact the expression and replication of the virus. The names, product size and functions of the genes of HTLV-1 are summarised in Table 1.
Table 1: Products of HTLV-1 genes

<table>
<thead>
<tr>
<th>5’ LTR</th>
<th>Contains regulatory elements essential for viral replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>gag</td>
<td>Group antigen nucleocapsid protein</td>
</tr>
<tr>
<td></td>
<td>p19 matrix</td>
</tr>
<tr>
<td></td>
<td>p24 capsid</td>
</tr>
<tr>
<td></td>
<td>p15 nucleocapsid</td>
</tr>
<tr>
<td>pol</td>
<td>Polymerase reverse transcriptase proteinase RT integrase</td>
</tr>
<tr>
<td></td>
<td>transcription of DNA from RNA splicing of protein precursors</td>
</tr>
<tr>
<td></td>
<td>synthesis of RNA integration of proviral DNA into host genome</td>
</tr>
<tr>
<td>env</td>
<td>Envelope Surface glycoprotein Transmembrane gp46 gp21</td>
</tr>
<tr>
<td></td>
<td>gp46 (SU) gp21 (TM)</td>
</tr>
<tr>
<td>pX</td>
<td>ORF I p12I ORF II p13I ORF III p30I ORF IV p40I</td>
</tr>
<tr>
<td></td>
<td>Rex Regulatory gene of ‘X’ region Cytoplasmic protein unknown function</td>
</tr>
<tr>
<td></td>
<td>Tax Transactivating gene of ‘X’ region</td>
</tr>
<tr>
<td></td>
<td>3’ LTR Contains regulatory elements essential for viral replication</td>
</tr>
</tbody>
</table>

The p12I protein, which is encoded by ORF I, is a small hydrophobic protein which localizes to the golgi and endoplasmic reticulum (ER) (Ding et al., 2001; Johnson et al., 2001; Albrecht & Lairmore, 2002). Although not necessary for HTLV-1 replication in vitro, p12I was shown to contribute to viral infectivity in vivo using a rabbit animal model system (Collins et al., 1999; Lairmore et al., 2000; Albrecht et al., 2002). Later studies have linked p12I to T cell activation. p12I has been shown to increase signal transducers and activators of the STAT5 pathway, increasing DNA binding and transcriptional activity in T cell lines as well as primary T cells (Nicot et al., 2001). In agreement with having a role in T cell activation/proliferation, p12I also interacts with both calnexin and calreticulin, ER--resident proteins which regulate calcium storage and increase calcium release (Ding et al., 2001).

The ORF II region of the viral mRNA encodes the p30 and p13 accessory proteins. When the p30 protein is expressed ectopically, it is found to localise to the nucleus and nucleus of transfected cells (Koralnik et al., 1993). The p13 represents the C-terminal 87 amino acids of p30. Ectopic expression of p13 localises it to the nucleus and mitochondria. While little is known about...