



**QR186.7 N17  
bltc C.1  
G379617**

THE BALME LIBRARY  
  
**3 0692 1007 3448 0**

**PREVALENCE OF ANTIBODIES TO HUMAN T-  
LYMPHOTROPIC VIRUS TYPE I AMONG  
BLOOD DONORS  
AT THE 37<sup>TH</sup> MILITARY  
HOSPITAL, ACCRA, GHANA.**

**BY**

**EDWIN GBLI NARTER-OLAGA**

**Pathology Department, University of Ghana Medical School, College of Health  
Sciences, Korle-Bu, Accra.**

**INTEGRI PROCEDAMUS**

**This thesis is submitted to the University of Ghana, Legon in partial fulfilment of the  
requirement for the award of M.Phil degree in Pathology**

**June 2004**

## DECLARATION

**The author carried out the work in this thesis alone unless otherwise indicated. Whenever the work of others is included, references are made to the source of information. This thesis has not in its present form or otherwise been submitted to this University for a degree, diploma, or other qualification.**



Edwin Gbli Narter-Olaga  
(Author)

*[Handwritten signature]*

Prof. Andrew A. Adjei  
(Supervisor)

*[Handwritten signature]*

Prof. Edwin K. Wiredu  
(Supervisor)

*[Handwritten signature]*

Prof. Yao Tettey  
(Supervisor)

*[Handwritten signature]*

Dr. Richard K. Gyasi  
(Supervisor)

*[Handwritten signature]*

Dr. (Brig. Gen.) Jaswant M. Wadhvani  
(Supervisor)

## ACKNOWLEDGEMENT

Without the Almighty God we cannot be alive to continue with His work of saving lives through science. I am sincerely grateful to all who contributed in diverse ways to help me enter the M. Phil programme, especially Dr. O. A. Duah, Head of the Department of Chemical Pathology, Korle-Bu. I wish to thank Prof. Andrew Anthony Adjei, Head of Medical Laboratory Science, School of Allied Health Sciences, College of Health Sciences, for his immeasurable assistance in this project. Many thanks also to my supervisors Prof. Edwin K. Wiredu, Dean, School of Allied Health Sciences, Korle-Bu, Prof. Y. Tettey, Head, Department of Pathology, Dr. Richard K. Gyasi, Department of Pathology, and Dr. (Brig. Gen.) Jaswant W. Wadhvani, Head of the Department of Morbid Anatomy, 37th Military Hospital. I am also grateful to all the Clinical Heads who are keen on making sure the M.Phil. programme is successful. I am also grateful to all the doctors in room 4 for tolerating my presence.

I must thank the secretaries in the departments of Pathology and Chemical Pathology for typing and printing some of my papers for me.

Finally to all the voluntary blood donors who consented to take part in the project, I render my Heart-felt thanks.

To all of you, I say a big THANK YOU.

## ABBREVIATIONS

AIDS=Acquired immune-deficiency syndrome

HIV= **H**uman **i**mmunodeficiency **v**irus

HTLV-1 = Human T- lymphotropic virus type-I

LAV= Lymphadenopathy associated virus

HCV= Hepatitis C virus

HBV= Hepatitis B virus

ATL=Adult T-cell leukaemia

CD =Cluster of differentiation

HAM=HTLV-1 associated myelopathy

RNA=Ribonucleic acid

DNA=Deoxyribonucleic acid

MHBTC=Military Hospital Blood Transfusion Centre

NBTS= National Blood Transfusion Service

RIBA= Recombinant immuno-blot assay

RIA = Radio immunoassay

EIA = Enzyme immunoassay



## TABLE OF CONTENT

### Title

Declaration

Acknowledgement

Abbreviations

Table of Contents

Chap 1 – Summary	.....	1 – 2
Chap 2 – Introduction	.....	3 – 6
Chap 3 - Literature Review	.....	7 – 17
Chap 4 - Diseases of HTLV Infections	.....	18 – 25
Chap 5 - Pathogenesis of HTLV Infections	.....	26 – 28
Chap 6 - Epidemiology of HTLV Infections	.....	29 – 33
Chap 7 - Diagnosis of HTLV Infection	.....	34 – 35
Chap 8 - Materials and Method	.....	36 – 39
Chap 9 – Results	.....	40 – 47
Chap 10 – Discussion	.....	48 – 50
Chap 11 – Conclusion	.....	51
Ethical Aspects	.....	52
References	.....	53 – 92
Appendix	.....	93 – 96

## CHAPTER 1

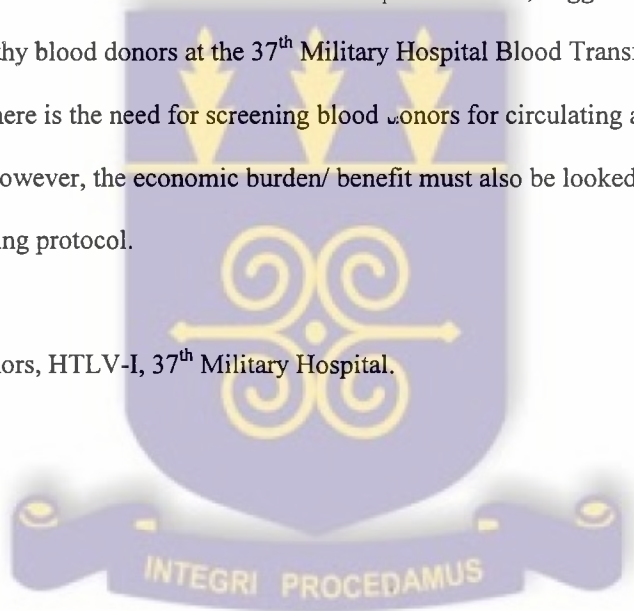
### SUMMARY

Several infectious diseases have been found to be associated with transfusion of **whole** blood or blood components. Reports from studies conducted in many African countries indicate a high incidence of blood-borne pathogens such as human T-lymphotropic virus type-I (HTLV-I) among healthy blood donors. Experimental data indicate that a major route for transmission of the HTLV-I is through blood transfusion. The prevalence of HTLV-I antibodies among blood donors in Ghana is not well documented. Population surveys cannot be conducted for financial reasons and therefore sentinel studies are the only means for providing information on the transmissions of infections such as HTLV-I, as well as monitoring the changes over time. The study was therefore undertaken to determine the prevalence of HTLV-I antibodies among blood donors, between the months of January to April 2004 at the 37<sup>th</sup> Military Hospital Blood Transfusion Service, Accra, Ghana. A combination of particle agglutination test and enzyme-linked immunosorbent assay (ELISA) was used to assess the prevalence and distribution of antibodies to HTLV-I. A structured questionnaire was also administered to the blood donors after an informed oral and written consent was taken. This involved questions on personal information, knowledge about HTLV-I transfusion, sexual behaviour, lifestyle and histories of transfusion-transmitted diseases.

Beginning from January to April 2004, blood samples were collected from blood donors, serum separated and analysed for the presence of antibodies to HTLV-I. A total of 1225 samples (1158 males and 67 females) were analysed. **Their ages ranged from 20-69 years; with majority (75.5%; 925/1225) of the blood donors studied between the 30-39 years age group.** Of the 1225 samples tested, 1196 were negative and 29 were positive for HTLV-I antibodies giving a prevalence rate of 2.4%. Two females were positive out of 67 (2.9%) and 27 males were positive out of 1158

(2.3%) male donors. Majority of the donors were married (914; 74.6%) and the rest (311; 25.4%) were not married. Of the married donors, 21 were positive for HTLV-I antibodies, giving a prevalence rate of 2.3% among married donor. **Most of the positive male donors were married with one wife (19; 65.5%), and one positive case had two wives (3.4%). Seroprevalence increased with marital status, suggesting marital status as the primary mode of transmission rather than number of wives.** There was no association of tattoo marks with HTLV-I infection ( $X^2 = 1.72$ ; or =2.07; 95% CI =0.16- 1.46). Knowledge about HTLV-I infection among blood donors was found to be very poor. Only 10 (0.82%) said they had heard of HTLV-I infection whilst 1215 (99.18%) had never heard about it. The results reported herein, suggest that HTLV-I is prevalent among healthy blood donors at the 37<sup>th</sup> Military Hospital Blood Transfusion Centre (MHBTC); and that there is the need for screening blood donors for circulating antibodies to HTLV-I infection. However, the economic burden/ benefit must also be looked at before including HTLV-I in the screening protocol.

Key words: blood donors, HTLV-I, 37<sup>th</sup> Military Hospital.



## CHAPTER 2

### INTRODUCTION

One important danger of blood transfusion is the transmission of blood borne infections. In the developed countries, during the past two decades, transfusion-transmitted infectious disease has become extremely rare because of improved donor selection processes, universal serologic screening of donors for blood-borne pathogens, and the shift from transfusion of fresh blood components to transfusion of refrigerated products (13,36). On the other hand, less developed countries, including Ghana, are not able to fully implement the above procedures to ensure safety of transfused donor blood. The demand for blood transfusion service in the less developed countries is high, because of high prevalence of infections that cause anaemia, malnutrition, the all too frequent road traffic accidents, and the surgical, and obstetric emergencies associated with blood loss (210).

The 37<sup>th</sup> Military Hospital Blood Transfusion Centre (MHBTC), Accra and National Blood Transfusion Service (NBTS), Accra, Ghana screen donor blood for human immunodeficiency virus (HIV) I & II, hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis. A strong case has not been made for screening for other blood-borne pathogens (such as human T-lymphotropic viruses) because of financial constraints that lead to failure to acquire enough test kits for serologic screening of other transfusion-associated pathogens and partly because of lack of technical support and interest by medical scientists and health authorities.

One of the infections that can be transmitted by blood transfusion is that caused by human T-lymphotropic virus type I (HTLV-I) (255). HTLV-I causes adult T-cell leukaemia and is common in Africa and among persons with African ancestry (289).

Reports from studies conducted in other African countries indicate high incidence of blood-borne pathogens such as HTLV-I, HCV and HBV among healthy blood donors (196,262,362). In a study conducted in Nigeria, Fleming *et al.* (66) found a prevalence of antibody to HTLV-I of 2.0% in Nigerian blood donors. Similarly, Sarkodie *et al.*, (285) in a recent study in Kumasi, Ghana, found the sero-prevalence of HTLV-I among blood donors to be 0.5%. In a related study, Lal *et al.* (163) reported a sero-prevalence rate of HTLV-I among urban and rural dwellers in southern Ghana to be 1-2%. More recently, Adjei *et al.* (2b) in a study conducted among healthy blood donors at the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana found the sero-prevalence to be 4.2%.

In a similar study conducted in Dar Es Salaam, Tanzania (196), 1% of the healthy subjects among the population studied had antibodies to HTLV-I. Similarly, Verdier *et al.* (349) in a study, conducted in La Cote d'Voire, found the sero-prevalence of antibodies to HTLV-I to be 3.5% in the general population. In Egypt, out of 14 patients fulfilling the diagnostic criteria for tropical spastic paraparesis (TSP) presenting to the Neurology Department of Cairo University, 2 (14.3%) were confirmed to be associated with HTLV-I (11). Reports from other studies suggest that HTLV-I infection is prevalent in other parts of Africa; and that the sero-prevalence rate of antibodies to HTLV-I in healthy African blood donors ranged from 0-9% and as high as 30% in several at risk groups (66,114,337). Prevalence rate of HTLV in South Africa is very low, about 0.01% among healthy blood donors (283).

In some of the technologically advanced countries such as Japan, Britain, United States of America donated blood and blood components are always screened for HTLV-I antibodies. In Ghana, because of financial reasons, screening of donated blood and blood components for other **transfusion-transmissible** pathogens cannot be done. Moreover, population surveys cannot be

conducted, and therefore sentinel studies are the only means for providing information regarding the transmissions of transfusion-transmitted infections as well as **for** monitoring changes over time.

## **OBJECTIVES**

The present study was therefore conducted to:

1. Determine the prevalence of antibodies to HTLV-I infection among blood donors **seen** at the MHBTC.
2. Determine **some** risk factors associated with HTLV-I infection.
3. **Evaluate the knowledge of HTLV-1 among blood donors in the general population.**

## **OUTPUT OF THE STUDY**

The study will provide the following outputs:

- Preliminary data on the prevalence of **HTLV-I infection in blood donors at the MHBTC, Accra, Ghana.**
- A description of the risk factors and mode of transmission of HTLV-I infection.

## **BENEFICIARIES OF THE STUDY**

The beneficiaries of the study will be primarily the Ministries of Defence and Health. The citizens of Ghana (especially blood donors and patients) will also benefit by having, a more accurate information on the prevalence and/or transmission of HTLV-I infections among blood donors.

## **JUSTIFICATION**

The proposed study aims at a better understanding of the mode of transmission of HTLV-I infection among blood donors and to create awareness of the need to evaluate the impact of HTLV-I on “walk-in” and voluntary blood donors at the MHBTC and surrounding communities. The relation between blood **transfusion** and high transmission of certain infections is an interesting field of

research that has not been explored in Ghana. Research into this area in detail could provide useful information as to the identification of blood donors who are carriers of or sero-positives for HTLV-I. The in-depth study of blood donors at the MHBTC might provide a better understanding of the risk factors for the transmission of such diseases. This information will be useful for advocacy to change life habits that put people at risk of developing the infection and provide a means of controlling transmission.



## CHAPTER 3

### LITERATURE REVIEW

#### History of Human T-cell Leukaemia Virus Discovery and Association with Disease

##### HUMAN T-cell Leukaemia virus type-I (HTLV-I)

HTLV-1 is an enveloped RNA virus and belongs to the retrovirus group. Four HTLV types were identified between 1977 and 1984. Type I is associated with adult T-cell leukaemia and tropical spastic paraparesis (HTLV-I-associated myelopathy). Type II is linked with hairy-cell leukaemia. Type III has been renamed HIV. Type IV is not yet linked to any human disease.

Adult T-cell leukaemia (ATL), was first described in 1977 by Takatsuki *et al* in Japan (342).

Since then, ATL has also been found in most parts of the world. The etiologic role for HTLV-I in ATL was established by:

- (a) seroepidemiologic surveys associating the virus with disease in patients with ATL and close contacts
- (b) the clonality of ATL tumour cells which correlates with monoclonal or oligoclonal pattern of integration of the viral genome in tumour cell DNA
- (c) infection of lymphocytes *in vitro*, resulting in immortalization of the same cell type (T-cells) as the tumour cell, and
- (d) HTLV-I being oncogenic in animal models.

HTLV-I was first identified in a lymphoblastoid cell line (HUT 102) established from a patient with a cutaneous T-cell lymphoma (257) and C type retrovirus particles were identified in this cell type. In retrospect, it is likely that this original patient had ATL. In 1981 it was shown that another cell line (MT-1) derived from a patient with ATL also harbored the virus and produced antigens that reacted with sera from patients with ATL (103,379). The viruses produced from these cell lines

were subsequently shown to be the same and were given the name HTLV I (259). Since the initial description of ATL and discovery of HTLV –I, the virus is now known to be associated with human disease other than ATL. The most notable of these is a neurologic disorder known as tropical spastic paraparesis (TSP) or more commonly HTLV –I associated myelopathy (HAM).

### **HUMAN T-CELL LEUKEMIA VIRUS TYPE- II**

HTLV –II was first identified in a T-cell line established from a patient with hairy-cell leukaemia (291). This cell line (Mo-1) was derived from splenic tissue in 1978 (292) and in 1982 was shown to harbor a retrovirus. Based on serologic cross-reactivity, this virus was shown to be related to (but distinct from) HTLV –I and was termed HTLV II (136). Like HTLV –I, HTLV II also, infects and immortalises T- lymphocytes *in vitro*. A few HTLV –II – infected patients have been characterised in detail, providing limited molecular evidence supporting an etiologic role of HTLV –II in a form of atypical hairy-cell leukaemia (276,277). The limited number of the individuals shown to harbor HTLV –II in association with specific diseases, has to date produced convincing epidemiological demonstration of a definitive etiologic role for HTLV –II in human malignancy.

### **HTLV-III AND HTLV-IV**

In April of 1984, Montagnier (211) and Gallo (260) independently identified a cytopathic retrovirus that was called lymphadenopathy associated virus (LAV) or human T-cell lymphotropic virus type –III (HTLV-III). This 100nm RNA virus now called human immunodeficiency virus (HIV), has an affinity for T-helper (T4) lymphocytes. The HIV virus is in the family Retroviridae (313).

Included in this family are oncoviruses such as HTLV-I and HTLV-II, which primarily induce proliferation of infected cells and formation of tumours. HIV is a lentivirus, a slow growing type of virus that causes chronic infection. The core of the virus contains an enzyme, reverse transcriptase that enables, the virus to copy the single stranded RNA into double stranded DNA (93). The genome of the virus is then integrated into the host DNA. The provirus can, through

transcription produce both viral genomic RNA and messenger RNA, which is translated into viral proteins. The virus is then, released by the host cell. In this process the host cell is often destroyed (93).

### GENETIC STRUCTURE.

The overall genetic structure of HTLV is similar to but distinct from that of other retrovirus.

(42,299)

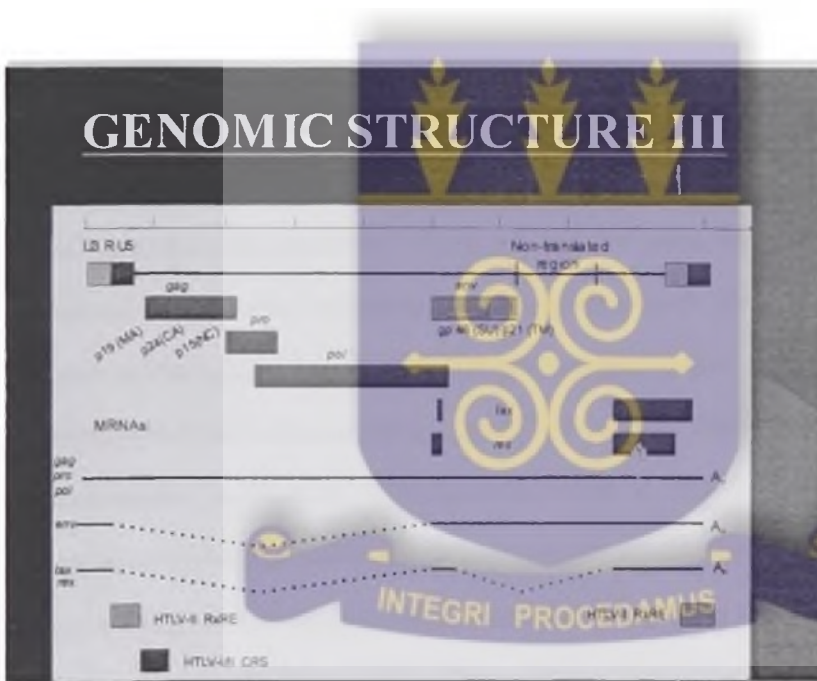


Figure 1

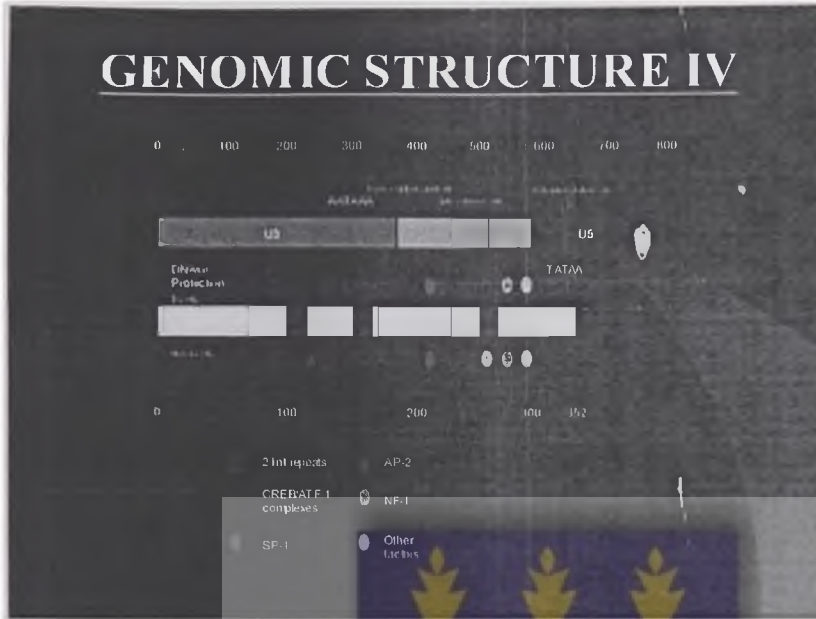


Figure 2

In addition to the usual complement of gag, pol, and env genes, there is a region at the 3' end of the genome not found in other replication – competent retroviruses. Because of its unknown function, this portion of the genome was initially referred to as the X region. Two genes have now been identified within the X region of HTLV I and II; the tax gene which encodes a protein responsible for transcriptional activation of the long terminal repeat (LTR) and a second gene, rex which is necessary for expression of structural proteins and partially overlaps tax in an alternative frame. Three messenger RNA (mRNA) species have been identified for HTLV (figure 1). Like other retroviruses, full length RNA is utilised for synthesis of gag and pol gene products and is also the genomic RNA packaged into virions. The primer used for reverse transcription of the genomic RNA is tRNA. A single- spliced subgenomic mRNA has two introns removed and encodes the tax and rex proteins. The tax gene initiation codon is the same as that used for the env gene and is contained within the second exon of the tax/rex mRNA. The initiation codon for the rex protein is also located within the second exon of this mRNA, a further 59 nucleotides upstream. Fig I above show the arrangements of the HTLV genome, genes, gene products and the three mRNA species.

## HTLV GENE PRODUCTS

### The gag Gene

As in other **retroviruses**, the gag region is initially translated as a polyprotein precursor, which is subsequently cleaved to form the mature gag polypeptides; the 19 kd matrix (MA), 24 – kd capsid (CA) and 15-kd nucleocapsid (NC) proteins (51, 95, 242). Like other retrovirus gag products, p19 is post-transcriptionally modified and contains myristic acid at the NH-terminus (203,242).

Myristoylation targets the p55 (gag) precursor polypeptide to the inner surface of the cell membrane (97). In other retroviruses, this has been shown to be important in the assembly and budding of virus particles from infected cells, and it is likely that this is also the case for HTLV.

### Protease

In HTLV, the protease is encoded by a reading frame which spans the 3' part of the gag region and 5' part of the pol region. Synthesis of the protease as part of the gag polyprotein precursor is accomplished by ribosomal frame shifting (225). In some molecular clones of HTLV – I, this protease open-reading frame contains stop codons (299), which may account for the lack of infectivity of these clones. Molecular clones have now been obtained which contain an intact protease, coding region (69,184,341). The function of the HTLV – 1 protease has been examined *in vitro*, and these studies have shown that (a) protease is responsible for processing the mature gag products and (b) autocatalyzed self- cleavage is responsible for generating the mature protease molecule (150,224,225).

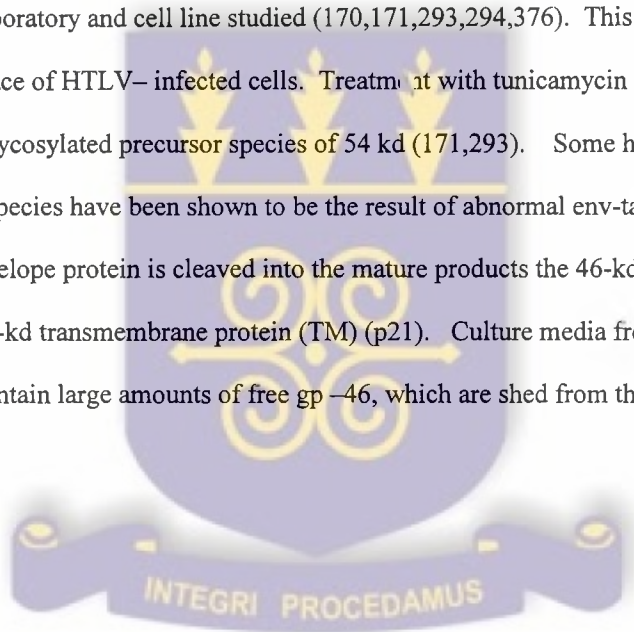
### The pol Region

The polymerase region is potentially able to encode an 896- amino acid product in HTLV-I and a 982 amino acid product in HTLV-II. However, a second ribosomal frame-shifting event is necessary to express the pol gene (225), resulting in polypeptides of 864 and 865 amino acids in

length for HTLV-I and HTLV –II respectively. The 5' portion of the pol gene is predicted to encode the reverse transcriptase protein and sequences further downstream the probable integrase and RNaseH functions. Human T-cell leukaemia virus reverse transcriptase, functions most efficiently using  $Mg^{2+}$  as the required divalent cation (257,265).

### **The env Gene**

The product of translation of the envelope open reading frame is a glycoprotein of 61 kd to 69 kd depending on the laboratory and cell line studied (170,171,293,294,376). This precursor protein is detected on the surface of HTLV– infected cells. Treatment with tunicamycin or endoglucosamine H results in a non-glycosylated precursor species of 54 kd (171,293). Some higher molecular weight env-related species have been shown to be the result of abnormal env-tax fusion proteins (202,333). The envelope protein is cleaved into the mature products the 46-kd surface glycoprotein (SU) (gp-46) and 21-kd transmembrane protein (TM) (p21). Culture media from HTLV – transformed cells contain large amounts of free gp –46, which are shed from the surface of the cells (291,373).



### **The tax Gene**

Two unique genes are found in HTLV, tax and rex (40,92,122,149,217,300,304,354,357) fig 1. Both genes are essential for viral replication (44). Antibodies against synthetic peptides provided definitive evidence that, proteins were encoded by these genes (82,172,202,311). The HTLV –II tax genes encode proteins of 40 and 37 kd, respectively. Both the 40-kd product of the HTLV-I tax gene and the 37-kd product of the HTLV-II tax gene are located primarily in the nucleus of the infected cells in the region defined as the nuclear matrix (82,172,310,311). The amino-terminal 48 residues of HTLV-1 tax protein comprise a nuclear localisation signal distinct from the highly basic

amino acid sequence, which performs this function in most nuclear proteins (312). Mutants of tax, which fail to localise to the nucleus, act as trans-dominant mutants of the wild-type protein.

The tax protein is a trans-acting transcriptional activator and increases the rate of transcription initiation from the promoter in the 5' LTR of the provirus genome. It was originally found that the transcription of the HTLV LTR was stimulated in virus-infected cells when compared with non-infected cells (42,314).

### **The rex Gene**

The rex gene encodes proteins of 27/21 kd for HTLV-1 and 26/24 kd for HTLV-II. Antisera directed against peptides from the carboxy-terminal portion of the rex open-reading frame immunoprecipitate both proteins (fig1).

### **Structure of the LTR**

The U3 region contains sequences that control transcription of the provirus (fig 2). In particular, three imperfect 21-nucleotide repeats are necessary for transacting transcriptional activation by tax (29,69,147,237,254,274,306). The U3 region also contains sequences responsible for termination and polyadenylation of mRNA's. The polyadenylation signal AATAAA, is an unusually long distance away from the polyadenylation site (~250 nucleotides) and is hypothesized to be brought into proximity with the polyadenylation site by secondary structure (3,16,306). The 5' part of the U3 region also encodes the carboxy terminus of the tax protein. Comparison of the LTRs of HTLV-I and HTLV-II reveals that those elements, which are found to be critical for viral gene expression (e.g. the 21-nucleotide repeats, polyadenylation signal and TATA box) are well conserved, whereas other regions are usually long in comparison to other retroviruses. These regions form the leader sequence encoded at the 5' end of the messenger RNAs (306).

The interaction of the HTLV LTR with proteins involved in regulation of transcription has been investigated (230,231), showing that multiple factors bind to different regions of the LTR in a complex pattern (fig 2). DNase-protected regions are evident over each of the three 21-nucleotide repeats, as well as other regions within U3, and binding of these factors is critical for regulation of HTLV transcription.

### **The Non-translated region**

A region of about 600 nucleotides is present between the end of the env gene and the beginning of the third exon of the tax and rex genes. The nucleotide homology of this region between HTLV-I and HTLV-II is relatively low compared to the rest of the genome. While there is an open-reading frame within this region in HTLV-I, in HTLV-II, there are termination codons in the equivalent reading frame. In an infectious clone of HTLV-II, deletion of approximately 300 nucleotides from within this region had no major deleterious effect on HTLV-II replication.

### **Genetic Variation of HTLV**

HTLV-I and HTLV-II share about 65% overall similarity at the nucleotide sequence level. The homology is lowered in the LTR and non-translated region and highest in the tax and rex genes. Among different HTLV-I isolates, there is remarkable sequences homogeneity. Isolates from Japan show 97% to 99% homology and even isolates obtained from geographically diverse regions such as the Caribbean, Japan and Africa show 96% to 99% homology (85,101,184,249,263,295,341). Indeed it has been suggested that sequencing HTLV-I isolates endemic in different races might be a means of monitoring the movement of ancient human populations, and might be useful in anthropologic studies (77). However, HTLV-I isolates from Malanesia show marked heterogeneity (approximately 7%) from the Japanese prototype of HTLV-

I, suggesting that HTLV-I may have originated in the Indo-Malay region rather than in Africa (75,80,302). Sequence homology among different HTLV-I isolates is equally high (167). However, it has been claimed that two distinct but closely related molecular subtypes of HTLV-II, designated HTLV-IIb, can be distinguished (58, 87). In spite of considerable effort, there is no indication that specific subtypes of HTLV are associated with particular pathologic consequences (52,146,151). The lack of genetic variability in HTLV may be due to the relatively low levels of viral replication in vitro. Replication of the provirus in dividing cells would result in a mutation frequency much lower than that of reverse transcription (57,174). In addition, cell associated replication i.e., passive replication of integrated viral genomes in dividing cells, may allow the HTLV to escape a degree of evolutionary pressure from antibody response. Compared to HIV, the HTLV, envelope genes, appears to be unable to tolerate many mutations without becoming non-functional. This might provide an additional basis for conservation of what might otherwise be a highly variable region of the virus genome (256).

## **HTLV INFECTIVITY**

### **Cells Susceptible to HTLV infection**

Direct cell-to-cell contact is usually required for efficient HTLV infection, although infection with cell-free virus preparation can occur (55, 279,301,368,372). In vitro infection of cells with HTLV is usually accomplished by co-cultivation of target cells with X-ray irradiated or mitomycin C-treated virus producing cells. By comparison with other retrovirus, infection by HTLV of susceptible cells is inefficient with a slow course. Usually only a small proportion of the cells express viral antigens, and this number may gradually increase over a course of weeks. The reason for this slow kinetics may relate to positive and negative regulation by tax/rex genes or other unknown processes of infection. Infection of human and or peripheral blood cells by HTLV-I/HTLV-II results in virus production and eventual immortalisation of the cells. Only T cells, have been shown to be transformed by HTLV. Cord and peripheral blood T cells of humans are

efficiently transformed. Productive infection with HTLV has only been demonstrated in a few cells of lymphoid origin (46, 107, 109, 113, 334, 357, 367, 381).

Conversely, HTLV-I appears to infect but not transform a number of different cell types in vivo. On several occasions Epstein Barr virus (EBV) –transformed B-cell lines productively infected with HTLV have been established from patients (1, 156, 375). B cells can also be infected in culture. There is also evidence that HTLV is capable of infecting immature cells from human bone marrow, which, do not have a T-cell phenotype (188). Infection of macrophages and cells of neural origin has been documented in vitro but not in vivo (4, 108, 157, 173, 266). Although cases of ATL with central nervous system (CNS) involvement are rare, direct infection of the CNS may account for such symptoms in a few patients (328). Ninety to 99% of HTLV-I DNA in peripheral blood from infected patients is found in CD4, CD8 cells (266). **In contrast, HTLV-I DNA is found predominantly in CD8 cells (116, 276).**

### **The HTLV Receptor**

The cellular receptor for HTLV has not been identified but has been demonstrated by various assays to exist on a wide variety of human and animal cells, including primate, canine, feline and rodent cells (47, 158, 220). The **presence** of a cell-surface receptor for HTLV has also been demonstrated by virus-induced cell fusion, leading to syncytium formation (110, 219). The absence of syncytium formation when HTLV-I and HTLV-II infected cells are mixed has been cited as evidence that the two viruses utilise the same receptor molecule, whereas the receptor for bovine leukaemia virus (BLV) is distinct (316, 317, 357)

### **T-cell Transformation by HTLV**

Both HTLV-I and HTLV-II will immortalise primary human peripheral blood T-cells in vitro (43, 45, 204, 258, 375). Transformation in this sense is defined as continuous cellular proliferation in

the absence of exogenous IL-2 and is distinct from oncogenesis. In addition to human T-cells T-lymphocytes **from** monkeys (205), rabbits (207), cats (111), and rats (335), have been transformed in vitro by HTLV-I.



## CHAPTER 4

### DISEASES OF HTLV-I INFECTIONS

#### ATL and HTLV-I

Several categories have been proposed as stages in ATL (143,145,191,309). These have been termed as follows:

- a) asymptomatic carriers state
- b) pre-leukaemic state (pre-ATL)
- c) chronic/smoldering ATL
- d) lymphoma type and
- e) acute ATL.

In some but not all cases, these categories may be temporarily related (140). The vast majority of HTLV-I-infected individuals are asymptomatic carriers for the virus (23, 83, 100, 103). These infected individuals are capable of transmitting the virus, since the proviral genome is integrated into host-cell DNA sequence (83). An infected individual has about 1% chance of developing a tumour over a lifetime of infection (153,154) although the cumulative lifetime chance of developing any HTLV-associated condition is slightly higher, 5% to 10% (56).

ATL generally occurs in adulthood, at least 20 to 30 years following infection (140). Some patients manifest a disorder known as pre-ATL, which is usually asymptomatic. Diagnosis of this condition was originally made by incidental detection of leukocytosis and/or

morphologically abnormal lymphocytes or more recently as a result of serological screening (143, 331). HTLV-I can be identified in the cells of those individuals by polymerase chain reaction amplification and the provirus, can be found to be integrated in abnormal T-lymphocytes in a monoclonal or oligoclonal pattern by Southern blot hybridisation. These findings indicated the beginning of the development of a clonal population of cells, some of which may progress to the malignancies found in acute ATL. Approximately 50% of patients with pre-ATL undergo spontaneous regression of lymphocytosis.

About 30% of patients with clinical manifestations of HTLV-I infections have chronic/smoldering ATL (140, 307, 370). These forms of ATL are less aggressive than the acute stage of the disease and are characterised by skin lesions, low levels of circulating leukaemic cells, and absence of visceral involvement. Patients in the smoldering ATL category present with skin lesions and marrow involvement, whereas patients with chronic ATL generally have elevated numbers of circulating ATL cells accounting for an increased leukocyte count. Patients in both the smoldering and chronic stages of ATL can progress into acute ATL within a period of months. Pre-ATL, smoldering ATL, and chronic ATL may represent transitional states in the development of the malignant clone that is evident in acute ATL.

In acute ATL (27, 33, 140, 307, 309), a dominant clone of malignant cells is present as evidenced by the process of:

- a) a single re-arrangement of T-cell antigen receptor gene (131, 192) and
- b) one or a few proviruses arranged in an oligoclonal fashion in the population of tumour cells (109, 298).

These patients have an elevated white blood cell count, with many morphologically abnormal T-lymphocytes having characteristic lobulated or flower-shaped nuclei. There is this also, often prominent eosinophilia and neutrophilia. Findings in acute ATL include lymphadenopathy that does not involve the mediastinum hepatosplenomegaly, and skin lesions due to infiltration of leukemic cells. **Interstitial** pneumonitis, also caused by leukemic cell infiltration, is sometime present. Patients manifest abnormalities in certain serum chemistries, including elevation of lactate dehydrogenase (LDH) hyperbilirubinaemia and hypercalcemia. Hypercalcaemia is associated with the presence of lytic bone lesions detected on the skull and long bones (28, 40, 89). Recent work has shown that hypercalcaemia in acute ATL may involve production of IL -1 $\alpha$  and parathyroid hormone-related protein by HTLV-I infected cells (61, 118, 119, 356). Patients with ATL are also immunocompromised and may present with opportunistic infections, including disseminated fungal infections, *Pneumocystis carinii* pneumoniasis, cytomegalovirus pneumonia, and bacterial infections (33, 38, 140, 344, 382). The only significant prognostic factor in acute ATL is the **presence** of ascites, which is associated with shorter survival. The median survival in acute ATL is approximately 6 months (39, 90, 129, 140,309), despite aggressive intervention with chemotherapy. The morphology of peripheral blood cells in ATL is variable. Typical ATL cells have a highly lobulated nucleus, but the degree of nuclear irregularity is variable (39, 232, 330). The histopathology of lymph nodes from patients with ATL is also heterogenous, but it most frequently falls into the large cell, immunoblastic classification (59, 90, 88, 129, 232). Nodal histopathology has no correlation with the patients' prognosis or therapy. Immunologic studies of ATL cells have revealed phenotypic heterogeneity for T-cell subset markers (213, 366). However, virtually all ATL cells have markers for CD4 antigen (39, 89, 94, 320), with rare exceptions having CD8 makers (30, 91). Although these markers usually indicate a helper/inducer phenotype, assays reveal that these cells have suppressor activity for immunoglobulin synthesis (197,328, 353, 367). Further studies suggested that this

suppression is not directly due to ATL cells but is instead, mediated via suppressor CD8 cells that are activated by the ATL cells (213). One characteristic marker of ATL tumour cells is high level of expression of the IL-2R-alpha chain (Tac antigen) (175, 342). The form of disease in some patients with ATL is predominantly that of a T-cell lymphoma rather than a leukaemia (38, 309, 369). These non-Hodgkins lymphomas have differing histologies and can involve any organ. Lymph node biopsy specimens from these patients contain oligoclonally integrated HTLV-1, indicating a clonal malignancy (370, 380). The differential diagnosis of ATL includes other T-cell malignancies such as non-Hodgkins' lymphoma, mycosis fungoides, Sezary syndrome, and T-cell chronic lymphocytic leukaemia. However, clearly, not all cases of these secondary conditions are associated with HTLV infection (37). A variety of laboratory studies can help establish a diagnosis of ATL, including, HTLV-1 serum positivity, hypercalcemia, elevated serum LDH, staining for terminal deoxynucleotidyl transferase (typically negative in ATL), and immunologic typing of ATL cells for CD4 and Tac antigen (IL-2R-alpha chain). **The definitive evidence** of disease is obtained if monoclonally or oligoclonally integrated HTLV-1 genome is present.

#### HTLV-1-ASSOCIATED MYELOPATHY

In 1985, a group of West Indian patients with a neurologic disease known as tropical spastic paraparesis (TSP) were found positive for HTLV-1 (79). Subsequently, a report from Japan described a number of patients with a slowly progressing myelopathy and pyramidal disturbances, all of whom had elevated HTLV-1 antibody titres (247). This neurologic disease was termed HTLV-1 associated myelopathy (HAM), now considered to identical to TSP.

Human T-cell leukaemia virus type 1-associated myelopathy has been described in all areas of the world known to be endemic for HTLV-1 (79,76, 179, 227, 246, 273, 349), and the risk factors are the same as those for HTLV infection (142). The lifetime risk for development of HAM has been estimated at less than 1% (138). Neurologic findings in HAM include weakness and spasticity of the extremities, hyper-reflexia, Babinski sign (either positive or negative), urinary/fecal incontinence, and mild peripheral sensory loss (244,349). The cerebrospinal fluid (CSF) of these patients contains anti-HTLV-1 antibodies and may show a lymphocytic pleocytosis, as well as elevated protein levels.

Patients with HAM generally have normal lymphocyte numbers, but morphologically atypical lymphocytes resembling ATL cells can be seen in peripheral blood or in the CSF (71, 243). Magnetic resonance imaging has demonstrated lesions in both the white matter and the paraventricular regions of the brains in patients with HAM (198,339, 349). Autopsy studies show significant abnormalities in the thoracic spinal cord, including demyelination, capillary proliferation, and perivascular cuffing with lymphocytic infiltration (5).

#### **OTHER DISEASES ASSOCIATED WITH HTLV-1**

A number of reports have suggested that hematologic malignancies other than ATL are associated with infection with HTLV-1 (78, 81, 86, 193, 290, 315, 318, 366). Cases of T-cell non-Hodgkin's lymphoma, T-prolymphocytic leukaemia, Sezary's syndrome, mycosis fungoides, small cell carcinoma and large granular lymphocytic leukaemia (T-gamma lymphoproliferative disease) have been described to be serologically or molecularly linked to HTLV-1 infection.

### **B-cell Chronic Lymphocytic Leukemia**

One malignancy distinct from ATL, in which HTLV-1 may play an indirect role occurs in certain cases of chronic lymphocytic leukaemia of B-cell origin (B-cell CLL). In Jamaica, a region in which HTLV-1 infection is endemic, HTLV seropositivity was significantly higher in patients with B-cell CLL than in the general population (25). When leukaemic cells of these patients were examined for HTLV-1, no integrated HTLV-1 provirus was found in the leukemic B-cell clones (48, 50). In two cases, hybridoma cell lines derived from the tumor cells of the B-cell CLL produced monoclonal antibodies that preferentially reacted with HTLV-1 proteins (185). It was proposed that the B-cell CLL is derived from the clonal outgrowth of a B-cell clone reactive against an HTLV-1 antigen. If B-cell CLL is related to HTLV-1 infection, then additional factors must also be involved.

### **IMMUNOSUPPRESSION**

There is some evidence for functional impairment of the cellular immune response in patients with ATL as well as among some HTLV-1 carriers (38, 100, 120). Defects in cell-mediated immunity may result in particular problems, with parasitic infections (227, 272). Immunosuppressive effects may lead to opportunistic infections (221, 227, 233). In this context, it is important to distinguish between patients who may be co-infected with HIV. Nevertheless, there is clear evidence that HTLV infection may result in some degree of immune system impairment (176,212, 215, 323).

### **CHRONIC INFLAMMATORY ARTHROPATHY**

Recent clinical reports have shown that chronic inflammatory arthropathy may occur in a few HTLV-1 infected individuals as a rare complication of infection (148, 229, 284). HTLV-1 infection is implicated in certain chronic arthritis in human (126).

### **HTLV-1 ASSOCIATED UVEITIS**

Studies in HTLV-1 endemic areas have indicated an association between HTLV-1 infection and an intra-ocular inflammatory disorder, uveitis. The sero-prevalence of HTLV in patients with uveitis without any other defined etiology is significantly higher than that in patients with non-uveitic ocular diseases (60,208,209,223).

### **HTLV - II DISEASE**

The first individual from whom HTLV – II was isolated had a disease termed hairy-cell leukaemia (136,291). Although most hairy-cell leukemiaias are of B-cell phenotype this patient's leukemic cells were T cells. The term atypical hairy-cell leukaemia is used to distinguish the disease from other T- and B-cell hairy cell leukemias, the majority of which are not associated with HTLV-II infection (276).

In 1985, a second HTLV-II infected patient was identified (277). Both patients shared the following features: T- cells lymphocytosis was evident: some circulating atypical lymphocytes were positive for tartrate-resistant acid phosphatase (TRAP) and had characteristic hairy cell leukaemia. A detailed analysis of HTLV-II with second patients provides evidence for an etiologic role of HTLV-II in this disease (276,277). There have now been several other reports of HTLV-II in association with T-cell malignancy (276,277).

One patient had T-cell CLL, characterised by marked neutropenia. Serologic evidence for HTLV-II infection has been obtained in populations by relatively crude enzyme-linked immunosorbent assays (ELISA) assays that distinguish between HTLV-I and HTLV-II antibodies. HTLV-II has been reported among intravenous drug abusers (IVDA) in Great Britain (336) and New York (271). The use of PCR has identified a high incidence of

HTLV-II infection among IVDA in New Orleans (167,278). CD8 T-cells elevation was observed in three of four HTLV-I infected IVDA.

Other complications of HTLV-II infection reported from diverse patient populations are highly reminiscent of HTLV-I associated diseases. These include spontaneous lymphocyte proliferation (363), mycosis fungoides (384), large granular lymphocyte leukaemia (180, 189), and neurologic complications similar if not identical to HAM (91,127,275,276).



## CHAPTER 5

### PATHOGENESIS OF HTLV INFECTIONS

#### ONCOGENESIS

When HTLV-1 infected lymphocytes are present in individuals at a sufficiently high proportions to allow detection of viral sequences by Southern blotting, the viral sequences are always present as integrated forms and are generally oligoclonal or monoclonal with respect to integrated sites (298,378). Initial infection by HTLV-1 results in transformation of a polyclonal population of cells and subsequent selection processes result in the evolution of clones, which may ultimately develop into malignant cells. Chromosomal abnormalities commonly exist in ATL cells (70,125,190,200,345,361) and the degree of cytogenetic aberration is often related to the severity of the disease, i.e. abnormalities are frequently found in acute ATL, but are less likely to be seen in chronic or smouldering ATL, suggesting the possibility of clonal evolution during disease progression. HTLV-transformation of T-lymphocytes results in a **pool** of proliferating cells which are not oncogenic themselves (64), but which provide a population from which a malignant clone may subsequently arise. This accounts for the long latent period between infection and manifestation of a tumor in HTLV disease. Thus T-cells transformation and oncogenesis are separate but related processes.

During the acute phase of ATL, when patients have an elevated leukocyte count with many malignant lymphocytes the viral DNA can be easily detected by southern blotting and is generally monoclonal with respect to integration sites. However, examinations of viral integration sites from different patients with ATL demonstrate that these are distinct and present on different chromosomes (298).

One of the distinguishing features of the virus in the acute phase and perhaps also in pre-ATL and chronic/smouldering ATL is that although the viral genome is present as a provirus in all of the tumor cells, there is no detectable expression of viral genes within the cells (39,83,264,338).

## **HTLV-ASSOCIATED MYELOPATHY**

The pathogenesis of HAM remains poorly understood. In contrast to ATL, which generally occurs 20 to 30 years following HTLV-I infection, HAM may develop in some patients within a few years following infection often after transfusion of infected blood (53,245). The presence of intrathecal antibody titers to HTLV-I indicates that the virus has infected the CNS in addition to the blood (105,244). Immune complexes have also been detected in the blood of patients with HAM (305). A number of studies have demonstrated the presence of HTLV-1 DNA in blood and lymphocytes of the CSF (19,380). It is noteworthy that polyclonal integration of the virus is detected in patients with HAM, as opposed to oligoclonal or monoclonal integration in ATL. Thus, development of HAM is not a consequence of the development of a malignant clone of cells. Characterisation of viral isolates from patients with HAM does not show any distinguishing features from HTLV-I from those with ATL and provides no evidence that a variant virus is involved in HAM (52,105, 129, 146, 151,341). Some authors have concluded that persistent active replication of HTLV-1 is an important factor in the pathogenesis of HAM (218), compared with the mostly quiescent state of the virus in ATL. Hypothetical mechanisms for the development of HAM are based upon autoimmune models. Primary demyelination and remyelination by oligodendrocytes occurs in the spinal cord lesions of HAM (234). Of note is the observation that patients with HAM tend to have higher levels of circulating antibody to HTLV-1 antigens than do those with ATL (244, 346). High levels of cytotoxic T-lymphocytes (CTLs), which predominantly recognise HTLV-1 infected cells, have been detected in patients with HAM (128,346), although this feature may not be unique for HAM (253).

### **HTLV-HIV CO-INFECTION**

Several epidemiologic surveys have indicated the existence of groups of individuals who are currently infected with both HTLV-I/II and HIV. This is particularly common among intravenous drug abusers (IVDA) who become infected by sharing contaminated needles and syringe, a major route of transmission of both viruses.

### **IMMUNE RESPONSE TO HTLV**

All patients with ATL make humoral antibodies to various HTLV-I antigens. The major viral gene products recognised by sera infected individuals are those of the gag, env and tax genes. As in all retroviruses, the gag proteins are the major immunogens and are responsible for the earliest antibodies to appear. Sera from infected individuals usually recognise all three gag proteins, p15, p24 and p19. There is considerable cross-reactivity between HTLV-I and HTLV-II, particularly in the region encoding p24 (134, 137, 267, 296, 297, 332, 373).

Serologic profile of HTLV- infected individuals varies considerably, including some individuals who display a virtually monospecific pattern of antibodies. Cellular immunity against HTLV- infected cells has also been described. Naturally, immunity to HTLV appears to be different from that of other retroviruses, in that human complement-mediated virolysis was not effective for HTLV virions, using either normal human serum or human serum from an HTLV antibody carrier. Thus the virus may have intrinsic resistance to humoral immune mechanisms (112).

## CHAPTER 6

### EPIDEMIOLOGY OF HTLV INFECTIONS

Human T-cell lymphoma virus infection was originally discovered in Japan, but has now been found in most parts of the world, including other areas in Asia, the Caribbean South America and Africa (21, 23, 40, 195, 289, 319, 352). Mapping the geographic distribution of HTLV-I/II has been complicated because conventional serologic approaches cannot distinguish between the two viruses. In general terms, HTLV-I predominates in southern Japan, the South Pacific, parts of West Africa and in African populations of the Western hemispheres, while HTLV-II clusters in Native American populations and among IVDA (183) the number of people around the world infected with HTVL-I has been estimated between 10 – 20 million (56). Sero-epidemiologic studies have been based upon a wide variety of assays, including ELISA's utilizing whole virion preparations (287, 322), immunofluorescence of fixed wells of HTLV-infected cell lines (100, 103) radioimmunoassays (336) western blots and radioimmunoprecipitation (170, 267,293,275). In Japan, an assay based on agglutination of gelatine particles has been used to screen all blood donors (117). In comparison to HIV infections the antibody titres for HTLV-I are relatively low (H. Lee, personal communication) and development of detectable antibodies may increase slowly with age (24, 102, 326).

#### HTLV –I IN JAPAN

The number of infected individuals in Japan has been estimated to be over 1 million (101) among a population of approximately 121 million. Rates of seropositivity in different regions vary widely 35% in Okinawa, 8% to 10% in Kyushi Province, and 0% to 1.2 % in nonendemic areas (102, 191,327). Even the incidence of HTLV-I infection in individual cities and locals within the

endemic regions is quite variable, probably due to the limited transmission of HTLV-I between socially isolated population centres (102, 155, 303,325).

Infection with HTLV-I, generally occurs quite early in life, probably perinatally (12, 98, 99, 222,327) but, it has been estimated that 30,000 to 50,000 Japanese have been infected through blood transfusions (283). In Japan, the primary modes of transmission within families are as follow:

- a) from male to female, via passage of HTLV-I infected lymphocytes in semen and
- b) from mother to child, primarily via lymphocytes in breast milk (12, 98,99). Female to male sexual transmission is also possible.

Many investigators have proposed hypothesis for the origin of HTLV-I in Japan. Some have theorised that **Roman** settlers who arrived in Japan between 300 and 1000 BC brought the virus to Japan (123). Other investigators have postulated that HTLV-I originated in Africa suggesting that Portuguese traders brought the virus to Japan in the 16th Century (66, 74)

## **HTLV-I IN OTHER COUNTRIES**

Human T-cell leukaemia virus type I is also endemic in

- a) other areas of Asia such as Taiwan, Okinawa
- b) the Caribbean basin, including northeastern South America and
- c) Central Africa (21, 23, 40, 195, 216, 269, 289, 319).

HTLV-1 infection and a few cases of ATL have been reported in Italy (85, 186), Israel (18) the Arctic (268), New Guinea (141) and the United States of America (26, 33). Adult T-cell leukaemia cases in Hawaii have been identified among Japanese Americans (24). The incidence of HTLV-1 infected individual appears to be increasing in Western Europe (336) and the United States of America particularly among IVDA and homosexuals (22, 25, 49, 65, 66, 73, 81, 114, 178, 240, 289,

358, 365). In one published study of IVDA in New York, a prevalence of 9%, 18% and 41% of HTLV-I, HTLV-II and HIV respectively was reported (271). In Trinidad, 15% of homosexuals were seropositive for HTLV-I, as opposed to 2.4% of the general population (17). Although HTLV-I is endemic in Trinidad and HIV was only relatively recently introduced into the country, approximately 40% of homosexuals were found to be infected with HIV, compared with less than 1% of the general population, suggesting that the same populations are at risk for infection with HTLV-1 and HIV, but that HTLV-I is spread less efficiently than HIV. Studies of HTLV-I/II among United States of America blood donors indicated that a significant proportion of blood samples are infected (169, 199). Human T-cell leukaemia virus seroprevalence is about three times greater than that for HIV-I (0.043% versus 0.013%); 52% of these cases are due to HTLV-II infections and 43% HTLV-I. up to 2,000 individuals per year may have been infected in the United States of America through blood transfusion before routine donor testing began (364). Some cases of transfusion-related ATL have also been reported (25,27, 31,33, 96). All blood supplies in the United States of America have been screened for HTLV-1 infection since 1988. In Europe, the general incidence of HTLV-1 infection appears to be lower than in the United States of America, although it is found at higher frequency in populations with known risk factors, in particular people of Caribbean origin and in IVDA (347). Human T-cell leukaemia virus infections, appears to be well established in IVDA in Italy (54, 85, 186). In the United Kingdom a recent survey of nearly 100,000 blood donors indicates an overall seroprevalence of 1:20,000 (30). A few European countries screen blood supplies for HTLV-1. In Africa HTLV-1 is found almost across the **whole of the continent**.

Reports from studies conducted in other African countries indicate high incidence of blood-borne pathogens such as HTLV-1, hepatitis C virus (HCV) and HBV among healthy blood donors (196,262,362). In a study conducted in Nigeria, Fleming *et al.* (65) found a prevalence of antibody to HTLV-I of 2.0% in Nigerian blood donors. Similarly, Sarkodie *et al.*, (285) in a recent study done in Kumasi, Ghana, found the sero-prevalence of HTLV-I among blood donors to be 0.5%. In

a related study Lal *et al.* (163) reported a sero-prevalence rate of HTLV-I among urban and rural dwellers in southern Ghana to be 1-2%. In a study conducted in Dar Es Salaam, Tanzania, (196), 1% of the healthy subjects among the population studied had antibodies to HTLV-I. Similarly, Verdier *et al.* (348) in a study conducted in La Cote d' Voire, found the seroprevalence of antibodies to HTLV-I to be 3.5% in the general population. Reports from other studies suggest that HTLV-I infection is prevalent in other parts of Africa; and that the sero-prevalence rate of antibodies to HTLV-I in healthy African blood donors ranged from 0-9% and as high as 30% in several at risk groups (65,114,337).

#### HTLV-II

HTLV-II infection appears to be **more common than previously thought** although distribution of the virus tends to be localised in certain population groups. HTLV-II infection is particularly high in IVDA (19, 54, 87, 106, 139, 161, 168). Although HTLV-II infection is extremely rare in Japan, certain ethnic groups elsewhere show higher incidence of **the** infection. This is particularly true for people of native, American origin and a high prevalence of **HTLV-II** infection **has been** identified in New Mexico.

#### TRANSMISSION OF HTLV

Human T-cell leukaemia virus type I transmission occurs through one of three different modes. First, mothers infected with HTLV-I can transmit the virus to the fetus or new born (144, 152, 326, 377). The mode of transmission **here** is either through transplacental passage of infected maternal lymphocytes or through infected lymphocytes in breast milk. The overall prevalence of HTLV-I among children born to infected mothers was 16%. The prevalence of HTLV-I among children breast-fed for over 3 months was significantly higher (27%) than that of those breast-fed for under 3 months. Of 78 bottle-fed children, 13% of children born to carrier mother are infected with HTLV-I

by routes other than breast milk (104). Polymerase chain reaction amplification had detected HTLV proviral DNA in the peripheral blood and milk of all carrier neonates, indicating that transplacental infection with HTLV-I is rare and that post-partum infection via breast milk is major perinatal transmission route (280, 281, 340). These observations have produced recommendations that carrier mothers should refrain from breast feeding in order to reduce the incidence of HTLV-I transmission to their offspring. Secondly, HTLV-I can be transmitted from male to female during sexual intercourse via HTLV-I infected cells in semen (222, 326). It is possible that female to male sexual transmission also occurs but only at a very low rate (32).

The third route of transmission is through infected blood and blood products. However, unlike HIV only blood products that involve passage of whole lymphocytes from donor to recipient can transmit the virus (132, 182, 199, 201, 239, 238, 307). A retrospective study of HTLV transmission via contaminated blood transfusion showed an apparent efficient transmission of 12% (321). This study concluded that transfusion transmission of HTLV-II to approximately 700 recipients per year occurred in the United States of America before routine donor testing began in 1988. In another study, antibodies were detected in 19 (0.3%) of 6,286 plasma donors from five regions of the United States of America but no HTLV-I/II antibodies were detected in hemophiliacs who were transfused regularly with non-inactivated plasma or its derivatives **emphasising** that the transfusion of HTLV-seropositive plasma products **do not** transmit the viral infection (36). However, HTLV has recently been transmitted extensively among IVDA presumably through passage of infected blood lymphocytes in shared needles. Thus the overall mode of HTLV-I transmission is similar to that of AIDS virus with the exception that the virus is apparently not readily transmitted by cell-free body fluids. In Africa the transmission of infected blood would increase because of the use of whole blood, which has not been leuco-depleted. Leuco-depletion actually deals with lymphocytes reduction in whole blood. Thus HTLV infection would be reduced.

## CHAPTER 7

### DIAGNOSIS OF HTLV INFECTION

#### Antibodies

Diagnosis of HTLV-I or HTLV-II infection requires both the ability to detect and discriminate between infections by either virus. Several methods have been utilised (102, 103, 117, 135, 199, 287, 322, 364, 373). The development of suitably rapid and sensitive assays has been complicated by the relatively low antibody titres in individuals with HTLV-I/II as compared to individuals with HIV. Several commercial assays based on ELISA or particle agglutination formats are now available for screening of HTLV-antibodies. An ELISA/agglutination assay is used as the primary screen, followed by confirmatory assays using Western blotting or radio immunoprecipitation. Screening of blood supplies since 1988 has reduced the overall rate of HTLV-I transmission via blood transfusion to an extremely low level although some HTLV-II infected blood is likely not to be detected by the assays currently available (10).

Serodia Fujirebio particle agglutination kit is available for screening of donated units before use. This kit has been used in Japan to detect antibodies to HTLV-1. The Serodia Fujirebio gelatine reagent is the most reliable in detecting HTLV antibodies in serum. Confirmatory tests by second, third and fourth generation ELISA kits have shown Serodia, Fujirebio gelatine particle kits to be sensitive in detecting antibodies to HTLV. Serodia Fujirebio gelatine kits, **detect** both antibodies to HTLV-I and HTLV-II. Serodia Fujibero gelatine particle uses two cells; sensitised and unsensitised cells.

The choice of Serodia gelatine particle as the reagent for the exercise is due to the fact that it is comparable to other test kits and is equally sensitive. The main complication in antibody screening

methods is an age-dependent increase in seropositivity, indicating that certain individuals may not develop antibodies until some time following infection (24, 48, 160, 235).

Detection of HTLV genetic material provides an alternative in patients with ATL, where the majority of lymphocytes harbor the provirus detection of this DNA by Southern blotting is relatively straight forward, and reliable. However, asymptomatic carriers are more problematic, since only a small proportion of cells are infected with the virus. One means formerly employed was to first cultivate the cells for 3 to 5 weeks, allowing:

- a) replication and spread of the virus and
- b) consequent amplification of viral genetic material. The virus can then be detected in these cultured cells by Southern hybridisation or in-situ hybridisation to HTLV RNA.

The **disadvantage** of this method is that *in vitro* culture of cells is time-consuming and expensive and is therefore not suitable as a rapid clinical screening assay. These problems have been solved by the application of PCR amplification of specific sequences in the virus genome (20, 60, 162). Polymerase chain reaction can be used to detect a single HTLV-I/II provirus and is now the method of choice for detection of HTLV DNA directly from blood and many other tissues. Commercial PCR kits for HTLV are available, however, the unmatched sensitivity of PCR also has a draw back. False positive results from inadvertent contamination of samples is a major problem. Target inactivation protocols are available to circumvent this problem in diagnostic laboratories. Although PCR is now a primary research tool, its use still presents problems of cost in large-scale screening **operations** particularly in underdeveloped countries where HTLV infection may be endemic.

## CHAPTER 8

### MATERIALS AND METHOD

*Study population.* Based on the assumption of a prevalence rate of 0.5% (282), a sample size of 1225 was calculated for a confidence interval of 95% and a power of 90% using standard methods. The study was carried out between the months of January and April 2004 among blood donors at the 37<sup>th</sup> MHBTC. The hospital is situated about six miles from KBTH, and it is a 600 bed hospital, which serves the military personnel, their dependants, surrounding urban population, and also referred cases from other military health posts outside Accra. In Ghana, blood donors are volunteers and are also sought from family members of patients and friends needing blood transfusion. Blood donors undergo clinical screening which involves a questionnaire (see **appendix**) and a routine medical examination; only those found to be healthy are bled. The criteria include checking the donor's blood pressure and pulse, physical examination for leprosy patches, tattoos, fungal elements and eczema and also checking the haemoglobin level. The haemoglobin level of male donor should be between 13.6g/dl to 18.6g/dl, that of the female must be between 12.0g/dl to 14.6g/dl. Questions are also asked about **the donor's** sexual behaviour and sexual preference.

Donated blood is routinely screened for HIV I & II antibodies, HBsAg, anti-HCV antibodies and **for** syphilis. In this study, additional blood was also taken from blood donors for detection of antibodies to HTLV-I.

*Sample collection.* Blood samples (about 3 ml) were collected from blood donors into 5 ml plain tubes. Serum was separated and kept at -20°C until analyzed. In addition, a structured questionnaire (Appendix A) was administered to the blood donors after an informed oral and written consent was taken.

*Serological Test.* Sera were screened for the presence of HTLV-I antibodies with a commercially available HTLV-I particle agglutination test kit and confirmed by ELISA (Serodia Fujirebio Inc., Japan) in accordance with the manufacturer's instructions. **The sensitivity and specificity of the assay are 100% and 98.5%, respectively.**

Principle of Passive Particle-Agglutination, test for Detection of Antibodies to HTLV-I.

Principle and Advantages:

The reagent is prepared **with** gelatin particles sensitized with HTLV-1 **antigen** on the principle that these sensitized particles **can be** agglutinated by anti-HTLV-1 antibody in human serum or plasma.

HTLV-1 is prepared by disrupting purified HTLV-1 **virus** with detergent. This is prepared by concentrating the culture fluid of a virus producing cell line; subjecting it to sucrose- gradient centrifugation, collecting the virus fraction corresponding to a density of about 1.16g/cm<sup>3</sup>.

Serodia HTLV-1 has the following advantage:

1. The test procedure is extremely simple as a microtitre technique and is particularly suitable for mass- screening of test samples.
2. The test is time- saving and results are readable by the naked eye after about 2 hours.
3. Serodia-HTLV-1 kit involves the use of a newly developed artificial carrier Fuji particle that does not show nonspecific agglutination usually observed with red cell carriers.

#### TEST PROCEDURE:

##### Preparation of Serum Specimens

Erythrocytes or other visible components present in the serum or plasma samples are removed by centrifugation prior to testing in order to preclude interference with test results. Inactivation of serum samples is not necessary.

1. 25ul of serum diluent was placed in wells 1 through 3 of a microtitre (U-shaped) plate

2. After centrifugation, 25ul of serum specimen was added to well 1 and mixed by filling and discharging the micropipette 3 or 4 times with fluid in well 1. 25ul of diluted solution well 1 was transferred into 2, mixed and 25ul again transferred into well 3. The procedure was repeated again in well 3 to obtain 2<sup>nd</sup> dilution.
3. After this 25ul of unsensitized cells were pipetted into well 2 and 25ul of sensitised cells was added to well 3 using the droppers supplied in kit.
4. The contents of the wells were thoroughly mixed using a tray mixer (automatic vibratory shaker).
5. The microtitre plates were then covered and placed on a level surface and allowed to stand at room temperature (15-25°C) for 2 hours.
6. The plates were read over a white sheet of paper.
7. Positive and negative controls were included and treated in a similar fashion.

#### PRINCIPLE OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzyme-linked immunosorbent assay, commonly known as ELISA or EIA, is similar in principle to radioimmunoassay but depends on an enzyme rather than a radioactive label. An enzyme conjugated to an antibody reacts with a colourless substrate to generate a coloured reaction product. The colour generated is proportional to the concentration of antigen or antibody present in the sample under test. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and p-nitrophenyl phosphatase.

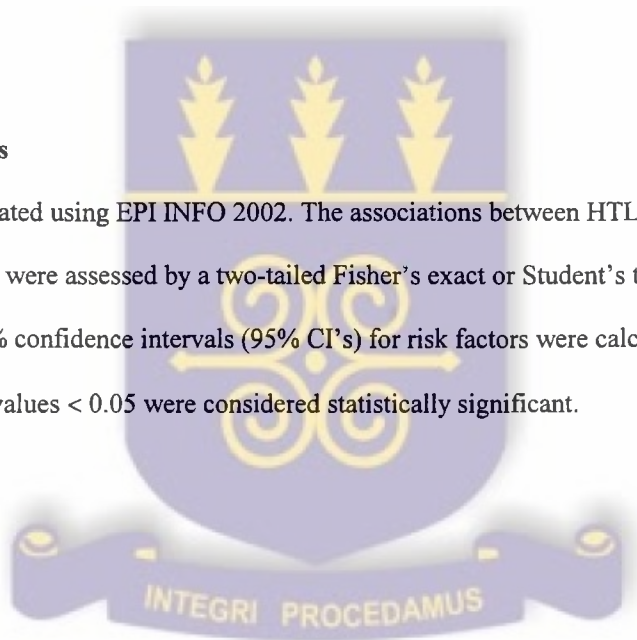
#### THE ELISA TEST PROCEDURE

The ELISA is based on a one step “sandwich” principle. Briefly, sera diluted 20-fold in phosphate buffered saline (PBS; pH 7.2) containing 0.05% Tween 20 and 20% goat serum were incubated overnight at 4°C in microtitre wells previously coated with a preparation of disrupted HTLV-1. After being washed with PBS-Tween, the wells were incubated for 1 hour at room

temperature with peroxidase-conjugated goat anti-human IgG appropriately diluted in PBS-Tween, containing 1% goat serum. Following washes with PBS-Tween and PBS, the wells were incubated for 20 minutes at room temperature with peroxidase substrate solution (which consists of Sorenson's phosphate citrate buffer, pH 5.0, containing 0.005% H<sub>2</sub>O<sub>2</sub> and 0.05%  $\sigma$ -phenylenediamine). The enzyme reaction was stopped, by adding 50ul 2M H<sub>2</sub>SO<sub>4</sub>, and the resulting colour was read on a Titertek plate reader at 492nm. All sera were assayed in duplicate and results were compared with those of standard HTLV-1 antibody positive and negative sera.

### **Statistical Analysis**

Statistics were calculated using EPI INFO 2002. The associations between HTLV-I and risk factors and covariates were assessed by a two-tailed Fisher's exact or Student's t-tests. Odds ratios (OR's) and 95% confidence intervals (95% CI's) for risk factors were calculated by a logistic regression model. P values < 0.05 were considered statistically significant.



## CHAPTER 9

### RESULTS

A total of 1225 donors, (1158 males and 67 females) were enrolled for the study (fig.1). Majority of the blood donors studied were in the **30-39 years age group forming 75.5% of the whole group** (Table 1). These 1225 donors had various educational backgrounds with 1006, forming 82.1%, having had at least some form of basic education (Table 2). Majority of the donors were traders (701; 57.2%) (Table 3) and were in the Hospital to donate blood for their relatives and friends. Of the 1225 donors, 311 (25.4%) were single and 914 (74.6%) were married (Table 4). Out of the 914 (74.6%) married donors, 43 (4.7%) had two wives and 871 (95.3%) had one wife (Table 4).

The donors were asked about the usage of contraceptive. Two (0.2%) did not use any form of **contraceptive**, whereas 1223 (99.8%) used some form of contraceptives (Table 5). Five (0.4%) used female condom, 42 (3.5%) did not use condom, and 1176 (96.0%) **sometimes** used male condoms (Table 5). The contraceptives used **included** male condom, female condom, **injectable** (Norplant) and oral contraceptive. The condoms were used as protection against sexually transmitted infections and not necessarily for the prevention of pregnancy. Injectable (Norplant) and oral contraceptive were for the prevention of pregnancy.

Of the 1225 donors, 1089 (88.9%) have donated blood before, while 136 (11.1%) were donating blood for the first time. Those that have donated blood before have done so between 1 and 7 times (Table 6). Also none of the donors had tattoo marks.

Knowledge about HTLV-1 infection among the blood donors was found to be very poor. Only 10 (0.82%) said they had heard of HTLV-1 infection whilst 1215 (99.18%) had never heard about it (Figure 2). **There was a statistically significant difference ( $P < 0.0001$ ) between them.**

**Table 2 – Educational Status of Blood Donors**

Type of Education	Frequency	Percentage (%)
Primary	130	10.61
Junior Secondary School	485	39.60
Middle school	391	31.92
Secondary school	100	8.16
Senior Secondary School	80	6.53
University	10	0.82
No schooling	27	2.20
Other	2	0.16
<b>Total</b>	<b>1225</b>	<b>100</b>

**Table 3 – Occupation of Blood Donors**

Occupation	Frequency	Percentage %
Accountant	1	0.1
Baker	10	0.8
Caterer	5	0.4
Catechist	2	0.2
Cleaner	14	1.1
Factory Hand	35	2.9
Hairdresser	8	0.6
Housewife	12	1.0
Messenger	18	1.5
Nurse	2	0.2
Seamstress	9	0.7
Salesgirl	5	0.4
Secretary	4	0.3
Social worker	1	0.1
Student	8	0.6
Tax officer	1	0.1
Teacher	87	7.1
Telephonist	2	0.2
Trader	701	57.2
Unemployed	300	24.5
<b>Total</b>	<b>1225</b>	<b>100</b>

sensitised, with HTLV-I antigen are agglutinated by anti-HTLV-I antibodies in human serum specimens. The sensitivity and specificity of the assay are 100% and 98.5%, respectively (251, 115). Positive results from this test only confirm previous exposure to HTLV-I and not necessarily active disease. This study highlights the need for screening blood donors for circulating antibodies to HTLV-I infection. This is especially important because of recent reports of close association between HIV and HTLV-I infections (177,196) although my study was not designed to explore such relationships.

The results of this study showed an overall prevalence rate of 2.4% of antibody to HTLV-I among blood donors at the MHTBC, Accra, Ghana. Study by *Lal, et al* showed a rate of 1-2% prevalence in urban and rural areas of Southern Ghana. The age distribution of HTLV-I positive donors ranged from 30-39, a group that has been described as being sexually most active and productive in terms of economic development and recovery. Although blood transfusion has been known as one of the major means of transmission of HTLV-I (182, 194), many HTLV-I positive donors did not admit to a past history of blood transfusion. This is an important finding because it means that though transfusion is a significant means of transmitting HTLV-I, attention must also be given to preventing HTLV-I infections from other sources other than blood transfusion. My observed increase in sero-prevalence of HTLV-1 with marital status (21 out of the 29 donors found to be HTLV-1 positive were married), points to marital status (vis-à-vis sexual contact) as the primary mode of transmission of HTLV-1. My sample of blood donors was largely comprised of males (1158 out of 1225 donors) and only 2 out of 67 screened female donors were positive for HTLV-I antibodies. The sero-prevalence was lower in males (2.33%; 27/1158) than females (2.99%; 2/67),  $P < 0.05$ .

The HTLV-I seroprevalence of 2.4% among the healthy blood donors in the current study was somewhat lower than the seroprevalence of 4.2% reported recently in healthy blood donors at the National Blood Transfusion Centre, Korle-Bu Teaching Hospital, Korle-Bu, Accra (2b). The difference cannot be discerned in this study but is probably due to the sample size (1225 in the current study versus 265 in reference 2b).

The present study, which is mainly descriptive was undertaken to investigate the prevalence of antibodies to HTLV-I among blood donors at the MHTBC. Despite this limitation, the general observation is that blood donated at MHTBC, Accra, contain relatively high prevalence of antibodies to HTLV-1. Further studies are in progress to determine the magnitude and the true prevalence using reverse transcriptase-polymerase chain reaction (RT-PCR). However, the prevalence rate reported herein is higher than those observed in Senegal (1.2%), and Liberia (1.6%) (216,360), and similar to the rate (2%) observed in Nigeria. Thus emphasizing the importance of

## **ETHICAL ASPECTS**

***Approval-*** The protocol for the study was submitted to and approved by the University of Ghana Medical School, Research and Ethical Committee. .

***Confidentially-*** All data was handled anonymously and confidentially.

***Safety Precautions-*** All materials were collected under maximum safety measures required for the handling of human tissues.



## REFERENCES

1. Aboud M, Golde DW, Wachsman W. Biology and leukemogenicity of human T-cell leukaemia viruses. In: Gallo RC, Haseltine W, Klein G, zur Hausen H, eds. *Viruses and human cancer; UCLA Symposium, Park City, Utah*. New York; Alan R. Liss; 1987:109-129
2. Adjei AA, Adiku TK, Ayeh-Kumi PF and Domfeh AB. Short Communications. Human T-lymphotropic Type-I Virus Specific Antibody Detected in sera of HIV/AIDS patients in Ghana. *Jpn J Infect. Diseases*, 2003; 56: 57 – 59.
- 2(b) Adjei AA, Adiku TK, Ayeh-Kumi PF, Armah H, Ansah J. Prevalence of Antibodies to Human T-lymphotropic Virus Type I Among Blood Donors in Accra, Ghana. *Ghana Medical Journal* 2003; 37: 133-136.
3. Ahmed YF, Hanly SM, Malim MH, Cullen BR, Greene WC. Structure-function analyses of the HTLV-I rex and HIV-I rev RNA response elements: insights into the mechanism of rex and rev action. *Genes Dev* 1990; 4: 1014 – 1022
4. Akagi T, Hoshida Y, Yoshino T, *et al.* Infectivity of human T-lymphotropic virus type I to human nervous tissue cells in vitro. *Acta Neuropathol* 1992; 84: 147 – 152.
5. Akizuki S, Nakazato O, Higuchi Y, *et al.* Necropsy findings in HTLV-1 associated myelopathy. *Lancet* 1987; 1:156-157.
6. Alexandre C, Charnay P, Verrier B. Transactivation of Krox-20 and Krox-24 promoters by the HTLV-1 tax through common regulatory elements. *Oncogene* 1991; 6: 51-57.
7. Alexandre C, Verrier B. Four regulatory elements in the human c-fos promoter mediate transactivation by HTLV-1 tax protein. *Oncogene* 1991; 6: 543-551.
8. Altman R, Harooch D, Garcia JA, Gaynor RB. Human T-cell leukaemia virus types I, and II exhibit different DNase I protection patterns. *J Virol* 1988; 62: 1339 – 1346.

9. Amagasaki T, Tomonaga T, Yamada Y, *et al.* Adult T-cell leukemia with an unusual phenotype. Leu – 2a positive and Leu – 3a negative. *Blut* 1985; 50:209- 211.
10. Association of American Blood Banks Technical Manual, Arlington, USA.
11. Amr Hassan, Azza Hassan, Ahmed El-Gohary, Mohammed El-Farrash and Fumio Kawano. HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis in Egypt. *Egypt J Neurol Psychiat Neurosurg* 1996; 33 (2): 181-185.
12. Ando Y, Nakano S, Saito K, *et al.* Transmission of adult T-cell leukaemia retrovirus (HTLV-1) from mother to child: comparison of bottle with breast-fed babies. *Jpn J Cancer Res* 1987; 78: 322-324.
13. Anonymous. Infectious disease testing for blood transfusion. NIH Consensus Statement 1995; 13:1-27.
14. Arya SK, Wong-Staal F, Gallo RC. T-cell growth factor gene: lack of expression in human T-cell leukaemia-lymphoma virus infected cells. *Science* 1984; 223: 1086-1087.
15. Azuma C, Tanabe T, Konishi M, *et al.* Cloning of cDNA for human T-cell replacing factor (IL-5) and comparison with the murine homologue. *Nucleic Acids Res* 1986; 14: 9149-9158.
16. BarShira A, Panet A, Honigman A. An RNA secondary structure juxtaposes two remote genetic signals for HTLV-I RNA 3'-end processing. *J Virol* 1991; 65: 5165 – 5173.
17. Bartholomew C, Saxinger WC, Clark JW, *et al.* Transmission of HTLV-1 and HIV among homosexual men in Trinidad. *JAMA* 1987; 257: 2604-2608.
18. Ben-Ishai Z, Haas M, Triglia D, *et al.* Human T-cell lymphotropic virus type-1 antibodies in Falashas and other ethnic groups in Israel. *Nature* 1985; 315: 665-666.
19. Berger JR, Svenningsson A, Raffanti S, Resnick I. Tropical spastic paraparesis-like illness occurring in a patient dually infected with HIV-I and HTLV-II. *Neurology* 1991; 41:5-7.

20. Bhagavati S, Ehlich G, Kula RW, *et al.* Detection of human T- cell lymphoma leukaemia virus type-1 DNA and antigen in spinal fluid and blood of patients with chronic progressive myelopathy. *N Engl J Med* 1988; 318:1141-1197.
21. Biggar RJ, Johnson BK, Oster C, *et al.* Regional variation in prevalence of antibody against human T-lymphotropic virus types 1 and II in Kenya, East Africa. *Int J Cancer* 1985; 35: 763-767.
22. Biggar RJ, Saxinger C, Gardiner C, *et al.* Type-1 HTLV antibody in urban and rural Ghana, West Africa. *Int J Cancer* 1984; 34:215-219.
23. Blattner WA, Kalyanaraman VS, Robert-Guroff M, *et al.* The human type-C retrovirus, HTLV, in blacks from the Caribbean region, and relationship to adult T-cell leukemia lymphoma. *Int J Cancer* 1982; 30: 257 – 264.
24. Blattner WA, Nomura A, Clark JW, *et al.* Modes of transmission and evidence for viral latency from studies of human T-cell lymphotropic virus type in Japanese migrant populations in Hawaii. *Proc Natl Acad Sci USA* 1986; 83:4892-4898.
25. Blattner WA, Saxinger C, Clark J, *et al.* Human T-cell leukemia lymphoma virus – associated lymphoreticular neoplasia in Jamaica. *Lancet* 1983; 2:61-64.
26. Blayney DW, Blattner WA, Robert-Guroff M, *et al.* The human T-cell leukemia lymphoma virus (HTLV) in South Eastern United States of America. *JAMA* 1983; 250:1048-1052.
27. Blayney DW, Jaffe ES, Blattner WA, *et al.* The human T-cell leukemia lymphoma virus associated with American adult T-cell leukemia lymphoma. *Blood* 1983; 62: 401– 405.
28. Blayney DW, Jaffe ES, Fisher RI, *et al.* The human T-cell leukemia lymphoma virus, lymphoma, lytic bone lesions and hypercalcemia. *Ann Intern Med* 1983; 98:144-151.
29. Brady J, Jeang KT, Durall J, Khoury G. Identification of the p40x – responsive regulatory sequences within the HTLV-I long terminal repeat. *J Virol* 1987; 61: 2173 – 2181.
30. Brennan M, Runganga J, Barbara JAJ, *et al.* Prevalence of antibodies to human T-cell leukaemia/lymphoma virus in blood donors in north London. *BMJ* 1993; 307: 1235-1239.

31. Broder, Bunn PA, Jaffe ES, *et al.* T-cell lymphoproliferative syndrome associated with human T-cell leukemia lymphoma virus. *Ann Intern Med* 1981; 100: 543-557.
32. Brodine SK, Oldfield EC III, Corwin AL, *et al.* HTLV-I among US marines stationed in a hyperendemic area: evidence of female-to-male sexual transmission. *J Acquir Immune Defic Syndr* 1992; 5:158-162.
33. Bunn PA Jr, Schechter GP, Jaffe E, *et al.* Clinical course of retrovirus associated adult T-cell lymphoma in the United States. *N Engl J Med* 1983; 309: 257 – 264.
34. Bunn PA Jr, Schechter GP, Jaffe E, *et al.* Clinical course of retrovirus associated adult T-cell lymphoma in the United States. *N Engl J Med* 1983; 309:257-266.
35. Burny A, Bruck C, Chantrenne H, *et al.* Bovine leukaemia virus: molecular biology and epidemiology, In: Klein G, ed. *Viral oncology*, New York: Raven Press; 1980:231-280.
36. Canavagio M, Leckie G, Allain JP, *et al.* The prevalence of antibody to HTLV-1/II in United States of America plasma donors and in United States of America and French hemophiliacs. *Transfusion* 1990; 30:780-782.
37. Capesius C, Saal F, Macro E, *et al.* No evidence for HTLV-1 infection in 24 cases of French and Portuguese mycosis fungoides and Sezary syndrome (as seen in France). *Leukemia* 1991; 5:416-419.
38. Cappell MS, Chow J. HTLV-1-associated-lymphoma involving the entire alimentary tract and presenting with an acquired immune deficiency. *Am J Med* 1987; 82:649-654.
39. Catovsky D, Matutes E, Btito-Babapulle V. Laboratory studies in Caribbean patients with adult T-cell leukemia-lymphoma. In: Gallo RC, *et al.*, eds. *Human T-cell leukaemia lymphoma*. Cold Spring Harbor Laboratory; 1984:275-284.
40. Catovsky D, Rose M, Goolden AWG, *et al.* Adult T-cell lymphoma-leukaemia 50(2) 50(3) in blacks from the West Indies. *Lancet* 1982; 1:639-647.
41. Centers for Disease Control. Licensing of screening tests for antibody to human T-lymphotrophic virus type I. *MMWR* 1988; 37: 736 – 747.

42. Chen ISY, McLaughlin J, Gasson JC, Clarke SC, Golde DW. Molecular characterization of genome of a novel human T-cell leukemia virus. *Nature* 1983; 305: 502 – 505.
43. Chen ISY, Quan SG, Golde DW. HTLV-I transforms normal human lymphocytes. *Proc Natl Acad Sci USA* 1983; 80: 7006 – 7009.
44. Chen ISY, Slamon DJ, Rosenblatt, Shah NP, Quan SG, Wachsman W. The x gene is essential for HTLV replication. *Science* 1985; 229: 54 – 58.
45. Chosa TN, Yamamoto Y, Tanaka Y, Koyanagi Y, Hinuma Y. Infectivity dissociated from transforming activity in a human retrovirus adult T-cell leukemia virus. *Gann* 1982; 73: 844 – 847.
46. Clapham P, Naggy K, Cheingson-Popov R, Weiss RA. Productive infection and cell-free transmission of human T-cell leukemia virus in a non-lymphoid cell line. *Science* 1983; 222: 1125 – 1127.
47. Clapham P, Nagy K, Weiss RA. Pseudotypes of Human T-cell leukemia virus types 1 and 2: neutralization of patients' sera. *Proc Natl. Acad Sci USA* 1984; 81: 3083 – 3086.
48. Clark JV, Saxinger C, Gibbs WN, *et al.* Seroepidemiology studies of human T- cell leukemia lymphoma virus type 1 in Jamaica. *Int J Cancer* 1985; 36:37-41.
49. Clark JW, Gurgo C, Franchini G, *et al.* Molecular epidemiology of HTLV-1 associated non-Hodgkin's lymphoma in Jamaica. *Cancer* 1988; 61:1477-1482.
50. Clark JW, Hahn BH, Mann DL, *et al.* Molecular and immunologic analysis of a chronic lymphocytic leukaemia case with antibodies against human T –cell leukemia virus. *Cancer* 1985; 56:495-499.
51. Copeland TD, Oroszlan S, Kalvanaraman VS, Sarngadharan MG, Gallo RC. Complete amino acid sequence of human T-cell leukemia virus structural protein p15. *FEBS Lett* 1983; 16: 390-395.
52. Daenke S, Nightingales S, Cruickshank JK, Boughamn CRM. Sequence variants of human T-cell lymphotropic virus type I from patients with tropical spastic paraparesis and adult T-

- cell leukemia do not distinguish neurological from leukemic isolates. *J Virol* 1990; 64: 1278 – 1282.
53. Delamarter RB, Carr J, Saxton EH. HTLV-1 viral-associated myelopathy after blood transfusion in a multiple trauma patient. *Clinic Orthop* 1990; 260: 191-194.
54. DeRossi A, Mammano F, DelMistro A, Chicco-Bianchi L. Serological and molecular evidence of infection by human T-cell lymphotropic virus type II in Italian drug addicts by use of synthetic peptides and polymerase chain reaction. *Eur J Cancer* 1991; 27:835-838.
55. DeRossi A, Aldovinini A, Franhini G, *et al.* Clonal selection of T lymphocytes infected by cell-free human T-cell leukemia/lymphoma virus type I: parameters of virus integration and expression. *Virology* 1985; 143: 640 – 645.
56. DeThe G, Bomford R. An HTLV-I vaccine: why, how for whom. *AIDS Res Hum Retroviruses* 1993; 9:381 – 386
57. Dougherty JP, Temin HM. High mutation rate of a spleen necrosis virus based retrovirus vector. *Mol Cell Biol* 1986; 6:4387 – 4395.
58. Dube DK, Sherman MP, Saksena NK, *et al.* Genetic heterogeneity in human T-cell leukemia/ lymphoma virus type II. *J Virol* 1993; 67: 1175 –1184. .
59. Duggan DB, Ehrlich GD, Darvey FP, *et al.* HTLV-1 induced lymphoma mimicking Hodgkin's disease: diagnosis by polymerase chain reaction amplification of specific HTLV-1 sequences in tumor DNA. *Blood* 1988; 71:1027-1032.
60. Eguchi K, Matsuoka N, Ida II, *et al.* Primary Sjogren's syndrome with antibodies to HTLV-1: clinical and laboratory features. *Ann Rheum Dis* 1992; 51:769-776.
61. Ejima E, Rosenblatt JD, Massari M, *et al.* Cell-type-specific transactivation of the parathyroid hormone related protein gene promoter by the HTLV-1 (HTLV-1) tax and HTLV-11 tax proteins. *Blood* 1993; 81:1017-1024.
62. Essex M, McLane MF, Tachibana N, Francis D, and Lee TH (1984). Seroepidemiology of T-cell leukaemia virus in relation to immunosuppression and the acquired immunodeficiency

- syndrome. p.355-362. In R.C. Gallo, M. Essex and L. Gross (eds), *Human T-cell lymphoma virus*. Cold Spring Harbour Laboratory, New York.
63. Felber BK, Paskalis H, Kleinman-Ewing C, Wong-Staal F, Pavlakis GN. The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. *Science* 1985; 229: 675 – 679.
64. Feuer G, Zack JA, Harrington WJ Jr, *et al.* Establishment of HTLV-1 T-cell lymphomas in severe combined immunodeficient mice. *Blood* 1993; 82: 722-731.
65. Fleming AF, Maharajan R, Abraham M, *et al.* Antibodies to HTLV-1 in Nigerian blood donors, their relatives, and patients with leukaemia, lymphomas and other diseases. *Int J Cancer* 1986; 38, 809 – 813.
66. Fleming AF, Maharajan R, Abraham M, Kulkarni AG, Bhusnurmath SR, Okpara, RA, Williams E, Akinsete I, Schneider J, Bayer U, Hunsmann G. Antibodies to ATL (HTLV) in Nigerian blood donors and patients with chronic lymphatic leukaemia or lymphoma. *Lancet* 1983; 2:334-335.
67. Francis DP, Curran TW and Essex M, (1983): Epidemic acquired immune deficiency syndrome (AIDS). Epidemiological evidence for a transmissible agent. *J Natl. Cancer Inst.* 71,1-4.
68. Fuji M, Niki T, Mori T, *et al.* HTLV-I tax induce expression of various immediate early serum responsive genes. *Oncogene* 1991; 6: 1023 – 1029.
69. Fujisawa J, Seiki M, Sata M, Yoshida M. A transcriptional enhancer sequence of HTLV-I is responsible for trans-activation mediated by p40 x 1 of HTLV-I. *EMBO J* 1986; 5:713 – 718.
70. Fukuhara S, Hinuma Y, Gotoh YI, Uchino H. Chromosome aberrations in T lymphocytes carrying adult T-cell leukaemia associated antigens (ATLA) from healthy adults. *Blood* 1983; 61: 205-207.

71. Furukawa Y, Fujisawa J, Osame M, *et al.* Frequent clonal proliferation of HTLV-1 (HTLV-1)-infected T-cells in HTLV-1 associated myelopathy (HAM-TSP). *Blood* 1992; 80:1012-1016.
72. Gallo R C, Mann D, and Broder S. (1982): Human T-cell leukemia-lymphoma virus is in T- but not B-lymphocytes from a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. U.S.A.*, 79, 5680-5683.
73. Gallo RC, Kalyanaraman VS, Sarngadharan MG, *et al.* Association of the human type C retrovirus with a sub set of adult T-cell Cancers. *Cancer Res* 1983; 43:3892-3899.
74. Gallo RC, Sliski A, Wong-Staal F. Origin of human T-cell leukaemia-lymphoma virus *Lancet* 1983; 2:962-963.
75. Gessain A, Boeri E, Yanagihara R, Gallo RC, Franchijni G. Complete nucleotide sequence of a highly divergent human T-cell leukemia (lymphotropic) virus type I (HTLV-I) variant from Melanesia: genetic and phylogenetic relationship to HTLV-I strains from other geographical regions. *J Virol* 1993; 1015 – 1023.
76. Gessain A, Francis H, Sonau T, *et al.* HTLV-1 and tropical spastic paraparesis in Africa. *Lancet* 1986; 2:698.
77. Gessain A, Gallo RC, Franchini G. Low degree of human T-cell leukemia/Lymphoma virus type-I genetic drift in vivo as a means of monitoring viral transmission and movement of ancient human populations. *J Virol* 1992; 66: 2288 – 2295.
78. Gessain A, Jovanelle A, Escarmant, P, Celender A, Schaffar Deshayes L, DeThe G. HTLV antibodies in patients with non-Hodgkin's lymphoma in Martinique. *Lancet* 1984; 1:1183-1184.
79. Gessain A, Vernaut JC, Maurs L, *et al.* Antibodies to human T-lymphotropic virus type – 1 in patients with tropical spastic paraparesis. *Lan et* 1985; 2:407-410.

80. Gessain A, Yanagihara R, Franchini G, *et al.* Highly divergent molecular variants of human T-cell lymphotropic virus type I from isolated populations in Papua New Guinea and Solomon Islands. *Proc Natl Acad Sci USA* 1991; 88: 7694 – 17698.
81. Gibbs WN, Lofters WS, Campbell M, *et al.* Non-Hodgkin lymphoma in Jamaica and its relation to adult T-cell. K. Lymphoma. *Ann Intern Med* 1987; 106:361- 368.
82. Goh WC, Sodroski J, Rosen C, Essex M, Haseltine WA. Sub-cellular localization of the product of the long open reading frame of HTLV-I. *Science* 1985; 227: 1227 -1228.
83. Gotoh YL, Sugamura K, Hinuma Y. Healthy carriers of a human retrovirus, adult T-cell leukemia virus (ATLV) demonstration by clonal culture of ATLV-carrying T-cells from peripheral blood. *Proc Natl Acad Sci USA* 1982; 79: 4780 – 4782.
84. Gradilone A, Zani M, Banllari G, *et al.* HTLV-1 and HIV infection in drug addicts in Italy. *Lancet* 1986; 2:753-754.
85. Gray GS, White M, Batman J, Mann D. Envelope gene sequence of HTLV-I isolate MT-2 and its comparison with other HTLV-1 isolates. *Virology* 1990; 177: 391 – 395.
86. Hall WW, Liu CR, Schneewind O, *et al.* Deleted HTLV-1 provirus in blood and cutaneous lesions of patients with mycosis fungoides. *Science* 1991; 253:317- 320.
87. Hall WW, Takahashi H, Liu C, *et al.* Multiple isolates and characteristics of HTLV-II. *J Virol* 1992; 66:2456-2463.
88. Hanaoka M, Sasaki M, Matsumoto, *et al.* Adult T-cell leukemia: histological classification and characteristics. *Acta Pathol Jpn* 1979; 29:723-738.
89. Hanaoka M, Takatsuki K, Shimoyama M, eds. Adult T-cell leukemia and related disease. *Gann Monogr Cancer Res* 1982; 28:1-237.
90. Hanaoka M. Clinical pathology of adult T-cell leukemia. *Nippon Ketsueki Gakkai Zasshi* 1981; 44:1420-1430.
91. Harrington WJ Jr, Sheremata W, Hjelle B, *et al.* Spastic ataxia associated with human T-cell lymphotropic virus type II infection. *Neurology* 1993; 33:411-414.

92. Haseltine WA, Sodroski J, Patarca R, Briggs D, Parkins D, Wong-Staal F. Structure of 3' terminal region of type II human T lymphotropic virus: evidence of new coding region. *Science* 1984; 225: 419 – 421.
93. Haseltine WA, Wong-Staal F. The molecular biology of AIDS virus. *Scientific American* 1988; 259 (4): 52-72.
94. Hattori S, Kiyokawa T, Imagawa K. *et al.* Identification of gag and env gene products of human T-cell leukemia virus (HTLV). *Virology* 1984; 136:338-347.
95. Hattori T, Uchiyama T, Toibana T, Takatsuki K, Uchino H. Surface phenotype of Japanese adult T-cell leukemia cells characterized by monoclonal antibodies. *Blood* 1985, 28: 645 – 617.
96. Haynes BF, Miller SE, Palker TJ, *et al.* Identification of human T-cell leukemia virus in a Japanese patient with adult T-cell leukemia and cutaneous lymphomatous vasculitis. *Proc Natl Acad Sci USA* 1983; 80:2054-2058.
97. Hayakawa T, Miyazaki T, Misumi Y, Kobayashi M, Fujisawa Y. Myristoylation-dependent membrane targeting and release of the HTLV-1 gag precursor, Pr53 (gag), in yeast. *Gene* 1992; 119: 273-277.
98. Hino S, Sugiyama H, Doi H, *et al.* Breaking the cycle of HTLV-1 transmission via carrier mothers' milk. *Lancet* 1987; 2: 158-159.
99. Hino S, Yamaguchi K, Katamine S, *et al.* Mother to child transmission of HTLV-1. *Gann* 1985; 76:474-480.
00. Hinuma Y, Chosa T, Komoda H, *et al.* Sporadic retrovirus (ATLV)- seropositive individuals outside Japan. *Lancet* 1983; 1: 824 – 825.
01. Hinuma Y. Sero-epidemiology of adult T-cell leukemia virus (HTLV-I/ATLV): origin of virus carriers in Japan. *AIDS Res* 1986; 517 – 522.

102. Hinuma Y, Komoda H, Chosa T, *et al.* Antibodies to adult T-cell leukaemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide seroepidemiologic study. *Int J Cancer* 1982; 29:631-635.
103. Hinuma Y, Nagata K, Hanaoka M, *et al.* Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl. Acad. Sci USA* 1981; 79:6476 – 6480.
104. Hirata M, Hayashi J, Noguchi A, *et al.* The effects of breast-feeding and presence of antibody to p40 (tax) protein of human T-cell lymphotropic virus type-I on mother to child transmission. *Int J Epidemiol* 1992; 21:989-994.
105. Hirose S, Uemura Y, Fushita M, *et al.* Isolation of HTLV-1 from cerebrospinal fluid of a patient with myelopathy. *Lancet* 1986; 2: 397-388.
106. Hjelle B, Scalf R, Swenson S. High frequency of human T cell leukemia-lymphoma virus type II infection in New Mexico blood donors: determination by sequence-specific oligonucleotide hybridization. *Blood* 1990; 76:450-454.
107. Ho DD, Rota TR, Hirsch MS. Infection of human endothelial cells by human T-lymphotropic virus type I. *Proc Natl. Acad Sci USA* 1984; 81: 7588 – 7590.
108. Hoffman PM, DhibJalbut S, Mikovits JA, *et al.* HTLV-I infection of monocytes and microglial cells in primary human cultures. *Proc Natl. Acad Sci USA* 1992; 89: 11784 – 11788.
109. Hoshino H, Esumi H, Miwa M, *et al.* Establishment and characterization of 10 cell lines derived from patients with adult T-cell leukemia. *Proc Natl Acad Sci. USA* 1983; 80: 6061 – 6065.
110. Hoshino H, Shimoyama M, Miwa M, Sugimura T. Detection of lymphocytes producing a human retrovirus associated with adult T-cell leukemia by syncytia induction assay. *Proc Natl Acad Sci U.S.A.* 1983; 80: 7337-7341.

111. Hoshino H, Tanaka H, Shimotohno K, *et al.* Immortalisation of peripheral blood lymphocytes of cats by human T-cell leukaemia virus. *In J cancer* 1984; 34: 513 – 517.
112. Hoshino H, Tanaka H, Miwa M, Okada H. Human T-cell virus is not lysed by human serum. *Nature* 1984; 310: 324-325.
113. Hoxie JA, Matthews DM, Cines DH. Infection of human endothelial cells by HTLV-I. *Proc Natl Acad Sci USA* 1984; 81: 7591 – 7595.
114. Hunsmann G, Bayer H, Schneider J, Schmitz H, Kern P, Dietrich M, Buttner DW, Goudeau AM, Kulkarni G, Fleming AF. Antibodies to ATL/HTLV-1 in Africa. *Med Microbiol Immunol* 1984; 173, 167-170.
115. Hunsmann G, Schneider J, Schmitt J, and Yamamoto N. (1983): Detection of serum antibodies to adult T-cell leukaemia virus and non -human primates and people in Africa. *Int. J Cancer* 32; 329-332.
116. Ijichi S., Ramundo MH, Takahashi H, Hall WW. In vivo cellular tropism of human T-cell leukemia virus type II (HTLV-II). *Exp Med* 1992; 176: 293 – 296.
117. Ikeda K, Fujino R, Matsui T, Yoshida T, Komoda H, Imai J. A new agglutination test for serum antibodies to adult T-cell leukaemia virus. *Gann* 1982; 75:845-848.
118. Ikeda K, Okazaki R, Inoue D, Ogata F, Matsumoto T. Transcription of the gene for parathyroid hormone-related peptide from the human is activated through a cAMP-dependent pathway by prostaglandin E1 in HTLV-1-infected T cells. *J Biol Chem* 1993; 268:1174-1179.
119. Ikeda K, Okazaki R, Inoue D, Ohino H, Ogata E, Matsumoto T. Interleukin-2 increases production and secretion of parathyroid hormone related peptide by human T-cell leukemia virus type1-infected T-cell possible role in hypercalcemia associated with adult T-cell leukemia. *Endocrinology* 1993; 132:2551-2556.
120. Imai J, Hinuma Y. Epstein-Barr virus-specific antibodies in patients with adult T-cell leukemia (ATL) and healthy ATL virus – carriers. *Int J Cancer* 1983; 31:197-200.

121. Inaba S, Sato H, Okochi K, *et al.* Prevention of transmission of human T-lymphotropic virus type I (HTLV-I) through transfusion by donor screening with antibody to the virus: one-year experience. *Transfusion* 1989; 29: 7 – 11.
122. Inoue J, Yoshida M, Seiki M. Transcriptional (p40x) and post-transcriptional (p27 – 11) regulators are required for the expression and replication of HTLV-I genes. *Proc Natl Acad Sci USA* 1987; 84: 3653 – 3657.
123. Ishida T, Hinuma Y. The origin of Japanese HTLV-1. *Nature* 1986;322 :504.
124. Isono T, Ogawa K, Seto A. Antiviral effect of zidovudine in the experimental model of adult T-cell leukaemia in rabbits. *Leuk Res* 1990; 14: 841-847.
125. Itoyama T, Sadamori N, Tokunaga S, *et al.* Cytogenetic studies of HTLV-1 carriers: a family study. *Cancer Genet Cytogenet* 1990; 49: 157-163.
126. Iwakura Y, Tosu M, Yoshoda E, *et al.* Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-1. *Science* 1991; 253: 1026-1028.
127. Jacobson S, Lehky T, Nishimura M, Robinson S, McFarlin DE, Dhibjalbut S. Isolation of HTLV-II from a patient chronic, progressive neurological disease clinically indistinguishable from HTLV-1-associated myelopathy/tropical spastic paraparesis. *Ann Neurol* 1993; 33: 392-396.
128. Jacobson S, Raine CS, Mingioli ES, Mcfarlin DE Isolation of an HTLV-1-like retrovirus from patients with tropical spastic paraparesis. *Nature* 1988; 331: 540-543.
129. Jaffe ES, Blattner WA, Blayney DW, *et al.* The pathologic spectrum of adult T-cell leukemia lymphoma in the United States. *Am J Surg. Pathol* 1984; 8:263-275.
130. Jarrett RF, Mitsuya H, Mann DI, *et al.* Configuration and expression of the T-cell receptor b-chain gene in human T-lymphotropic virus infected cells. *J Exp Med* 1986; 163: 383 – 399.
131. Jarrett RF, Mitsuya H, Mann DI, *et al.* Configuration and expression of the T-cell receptor b-chain gene in human T lymphotropic virus infected cells. *J Exp Med* 1986; 163:383-390.

132. Jason JM, McDougal JS, Cabradilla C, Kalyanaram VS, Evatt BL. Human T- cell leukemia virus (HTLV-I) p24 antibody in New York City blood product recipients. *Am J Hematol* 1985; 20:129-137.
133. Kalland KH, Langhoff E, Bos HJ, Gottlinger H, Haseltine WA. Rex-dependent nucleolar accumulation of HTLV-1 mRNAs. *New Biologist* 1991; 3: 93-97.
134. Kalyanarama VS, Jarvis-Morar M, Sarngadharan MG, Gallo RC. Immunological characterization of the low molecular weight *gag gene* proteins p19 and p15 of human T-cell leukaemia-lymphoma virus (HTLV) and demonstration of human natural antibodies to them. *Virology* 1984; 132:61-70.
135. Kalyanarama VS, Sarngadharan MG, Nakao Y, Ito Y, Aoki T, Gallo RC. Natural antibodies to the structural core protein (p24) of the human T-cell leukaemia-lymphoma retrovirus found in sera of leukaemia patients in Japan. *Proc Natl Acad Sci USA* 1982; 79: 1653-1657.
136. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, *et al.* A new subtype of human T-cell leukaemia virus (HTLV-II) associated with T-cell variant of hairy cell leukaemia. *Science*. 1982; 218: 571 – 573.
137. Kalyanarama VS, Sarngadharan MG, Poiesz B, Ruscetti FW, Gallo RC. Immunological properties of a type C retrovirus isolated from cultured human T-lymphoma cells and comparison to other mammalian retroviruses. *J Virol* 1981; 38:906-915
138. Kaplan JE, Osame M, Kubota H, *et al.* The risk of development of HTLV-1-associated myelopathy/tropical spastic paraparesis among persons infected with HTLV-1. *J Acquir Immune Defic Syndr* 1990; 3: 1096-1101.
139. Kaplan MH, Hall WW, Susin M, *et al.* Syndrome of severe skin disease, eosinophilia, and dermatopathic lymphadenopathy in patients with HTLV-II complicating human immunodeficiency virus infection. *Am J Med* 1991; 300-309.

140. Kawano I, Yamaguchi K, Nishimura H, Tsuda II, Takatsuki K. Variation in the clinical courses of adult T-cell leukemia. *Cancer* 1985; 55: 851 – 856.
141. Kazura JW, Saxinger WC, Wenger J, *et al.* Epidemiology of HTLV-1 infection in East Sepik Province, Papua New Guinea. *J Infect Dis* 1987; 155:1100-1107.
142. Khabbaz R, Keplan J, Osame M, *et al.* Nationwide survey of HTLV-1- associated myelopathy in Japan: association with blood transfusion. *Ann Neurol* 1990; 28:50-56.
143. Kinoshita K, Amagasaki T, Ikeda S, *et al.* Preleukemic state of adult T-cell leukemia: abnormal T lymphocytosis induced by human adult T-cell leukemia-lymphoma virus. *Blood* 1985; 6:120-127.
144. Kinoshita K, Hino S, Amagasaki T, *et al.* Demonstration of adult T-cell leukemia virus antigen in milk from three seropositive mothers. *Gann* 1984; 75:103-105.
145. Kinoshita K, Hinos S, Amagasaki T. Development of adult T-cell leukemia-lymphoma (ATL) in two anti-ATL associated antigen-positive healthy adults. *Gann* 1982; 73: 684 – 685.
146. Kinoshita T, Tsuimoto A, Shimotohno K. Sequence variations in LTR and env regions of HTLV-1 do not discriminate between the virus from patients with HTLV-1-associated myelopathy and adult T-cell leukemia. *Int J Cancer* 1991; 47: 491-495.
147. Kitado H, Chen ISY, Shah NP, Cann AJ, Shimotohno K, Fan H. U3 sequences from the HTLV-I and -II LTR's confer pX protein responsiveness to a murine leukemia virus LTR. *Science* 1987; 235: 901 – 904.
148. Kitajima I, Yamamoto K, Sato K, *et al.* Detection of human T-cell lymphotropic virus type 1 proviral DNA and its gene expression in synovial cells in chronic inflammatory anttropy. *J Clin Invest* 1991; 88:1315-1322.
149. Kiyokawa T, Seiki M, Iwashita S, Imagawa K, Shimizu F, Yoshida M. P27 x 111 and p21 x 111, proteins encoded by the pX sequence of HTLV-I. *Proc Natl Acad Sci USA* 1985; 82: 8359 – 8363.

150. Kobayashi N, Ohi Y, Asano T, *et al.* Purification and characterization of HTLV-I protease produced in *Escherichia coli*. *FEBS Lett* 199; 293: 106 – 110.
151. Komurian F, Pelloguin F, DeThe G. In vivo genomic variability of HTLV-I depends more upon geography than upon pathologies. *J Virol* 1991; 65: 3770 – 3778.
152. Komuro A, Hayami M, Fujii H, Miyahara S, Hirayama M. Vertical transmission of adult T-cell leukaemia virus. *Lancet* 1983; 1:240.
153. Kondo T, Kono H, Nonaka H, *et al.* Risk of adult T-cell leukemia lymphoma in HTLV-I carriers. *Lancet* 1987; 2:159.
154. Kondo T, Nonaka H, Kono H, Miyamoto N, Hanaoka M. Epidemiology of ATLL and preceding stage in a cluster area. *Recent Adv Res* 1987; 25:156 – 161.
155. Kondo T, Nonaka H, Miyamoto N, *et al.* Incidence of adult T-cell leukaemia-lymphoma and familial clustering. *Int J Cancer* 1985; 35: 749-751.
156. Koyanagi Y, Yamamoto N, Kobayashi N, *et al.* Characterization of human B-cell lines harbouring both adult T-cell leukaemia (ATL) virus and Epstein-Barr virus derived from ATL patients. *J Gen Virol* 1984; 65: 1781-1789.
157. Koralnik IJ, Lemp JF Jr, Gallo RC, Franchini G. In vitro infection of human macrophages by human T-cell leukemia/lymphotropic virus type I (HTLV-I). *AIDS Res Hum Retroviruses* 1992; 8: 1845-1849.
158. Krichbaum-Stenger K, Poiesz B, Keller P, *et al.* Specific adsorption of HTLV-1 to various target human and animal cells. *Blood* 1987; 70: 1303-1311.
159. Kushida S, Matsumura M, Tanaka H, *et al.* HTLV-1-associated myelopathy/tropical spastic paraparesis-like rats by intravenous injection of HTLV-1-producing rabbit or human T-cell line into adult WKA rats. *Jpn J Cancer Res* 1993; 84:831-833.
160. Kusuhara K, Sunoda S, Takahashi K, Tokugawa K, Fukushige J, Ueda K. Mother to child transmission of HTLV-1 (HTLV-1) a fifteen-year follow-up study in Okinawa, Japan. *Int J Cancer* 1987; 40: 755-757.

161. Kwok S, Gallo D, Hanson C, McKinney N, Poiesz B, Sninsky JJ. High prevalence of HTLV-II among intravenous drug abusers: PCR confirmation and typing. *AIDS Res Hum Retroviruses* 1990; 6:561-565.
162. Kwok S, Mack DH, Mullis KB, *et al.* Identification of human immunodeficiency virus sequences by using in vitro enzymatic amplification and oligomer cleavage detection. *J Virol* 1987; 61:1690-1694.
163. Lal RB, Owen SM, Mingle J, Levine PH, Manns A. Presence of human T-lymphotropic virus types-I and-II, in Ghana, West Africa. *AIDS Res Hum Retroviruses* 1994; 10, 1747 – 1750.
164. Landau NR, Page KA, Littman DR. Pseudotyping with HTLV-I broadens the human immunodeficiency virus host range. *J Virol* 1991; 65:162-169.
165. Larouze B, Schaffar-Deshayes L, Blesonski S, Gaudebout C, Afoutou JM, Couillin P, Da Graca PM, Diakhate L, Frelut MI, Jeddi M, Mercier E, Noireau F, Nouasria B, Ravaoarino M, Levy JP. Antibodies to HTLV-1 p24 in African and Portuguese populations. *Cancer Res* 1985; 45. 4630 – 4632.
166. Lee HH, Idler KB, Swanson P, *et al.* Complete nucleotide sequence of HTLV-II isolate NRA: comparison of envelope-sequence variation of HTLV-II isolates from U.S.A. blood donors and U.S.A. and Italian IV drug users. *Virology* 1993; 196: 57-69.
167. Lee HH, Swanson P, Shorty VS, Zack JA, Rosenblatt JD, Chen ISY. High rate of HTLV-II infection in seropositive IV drug abusers in New Orleans. *Science* 1989; 244: 471 – 475.
168. Lee HH, Weiss SH, Brown LS, *et al.* Patterns of HIV-1 and HTLV-I/II in intravenous drug abusers from the Middle Atlantic and Central Regions of the U.S.A. *J Infect Dis* 1990; 162:347-352.
169. Lee HH, Swanson P, Rosenblatt JD, *et al.* Relative prevalence and risk factors of HTLV-I and HTLV-II infection in US blood donors. *Lancet* 1991; 337:1435-1439.

170. Lee TH, Coligan JE, Homma T, McLane MF, Tachibana M, Essex M. Human T-cell leukemia virus-associated membrane antigen (HTLV-MA): identity of the major antigens recognized after virus infection. *Proc Natl Acad Sci USA* 1984; 81: 3856 – 3860.
171. Lee TH, Coligan JE, Sodroki JG, *et al.* Serological cross-reactivity between envelope gene products of type I and type II human T-cell leukemia virus. *Proc Natl Acad Sci USA*. 1984; 81: 7579 – 7583.
172. Lee TH, Coligan JE, Sodroski JG, *et al.* Antigens encoded by 3' terminal region of human T-cell leukemia virus; evidence for a functional gene. *Science* 1984; 226: 57 – 61.
173. Lehky TJ, Cowan EP, Lampson LA, Jacobson S. Induction of HLA Class I and Class II expression in human T-lymphotropic virus type I – infected neuroblastoma cells. *J Virol* 1994; 68: 1854 – 1863.
174. Leider JM, Palese P, Smith FI. Determination of the mutation rate of a retrovirus. *J Virol* 1988; 62: 3084 – 3091.
175. Leonard WJ, Depper JM, Uchiyama T, *et al.* A monoclonal antibody that appears to reorganize the receptor for human T-cell growth factor: partial characterization of the receptor. *Nature* 1983; 300:267-269.
176. Levy J, Mascart-Lemone F, Song M, Dobec M. Immunosuppression by HTLV-1 infection (II). *Lancet* 1991; 337:309.
177. Levy JA. (1994) : Revised classification system for HIV infection and expanded AIDS surveillance case definition for adolescents and adults. p. 241-246. HIV and pathogenesis of AIDS. *American Society of Microbiology, Washington, D.C.*
178. Lofters WS, Gibbs WN, Campbell M, Morgan OS, LaGrenade L, Hanchard B. Clinical features of non-Hodgkin's lymphoma in Jamaica. *West Indian Med J* 1984; 33:171-175.
179. Lolli F, Fredrikson S, Kam-Hanseu S, Link H. Increased reactivity to HTLV-1 in inflammatory nervous system diseases. *Ann Neurol* 1987; 22:67-71.

180. Loughran TP Jr, Coyle T, Sherman MP, Starkebaum G, Ehrlich GD, Ruscetti FW, Poiesz BJ. Detection of human T-cell leukaemia/lymphoma virus, type-II, in a patient with large granular lymphocyte leukaemia. *Blood* 1992; 80: 1116-1119.
181. Lusso P, Lori F, Gallo RC. CD4-independent infection by human immunodeficiency virus type I after phenotypic mixing with human T-cell leukaemia viruses. *J Virol* 1990; 64:6341-6344.
182. Maeda Y, Furukawa M, Takehara Y, *et al.* Prevalence of possible adult T-cell leukemia virus-carriers among volunteer blood donors in Japan: a nation wide study. *Int J Cancer* 1984; 33:717-720.
183. Madeleine MM, Wiktor SZ, Goedert JJ, *et al.* HTLV-I and HTLV-II, worldwide distribution: reanalysis of 4,832 immunoblot results. *Int J Cancer* 1993; 54: 255-260.
184. Malik KTA, Evoir J, Karpas A. Molecular cloning and complete nucleotide sequence of an adult T-cell leukemia virus/HTLV-I (ATL/HTL-I) isolate of Caribbean origin: relationship to other members of the ATL/HTLV-I subgroup. *J Gen Virol* 1985; 69: 1695 – 1710.
185. Mann D, Desantis P, Mark G, *et al.* HTLV-1 associated B-cell CLL: indirect role for retrovirus in leukemogenesis. *Science* 1987; 236:1103-1106.
186. Manzari V, Gradilone A, Barillary G, *et al.* HTLV-1 is endemic in Southern Italy: detection of the first infectious cluster in a white population. *Int J Cancer* 1988; 36:557-559.
187. Markham P D, Salahuddin S Z, Kalyanaraman VS, Popovic M, Sarin PS and Gallo R C. (1983): Infection and transformation of fresh human umbilical cord blood cells by multiple sources of human T-cell leukaemia lymphoma virus (HTLV). *Int. J. Cancer* 31, 413-420.
188. Markham PD, Salahuddin SZ, Robert-Guroff M, Gallo RC. Transformation of different phenotypes of human T-lymphocytes from bone marrow by HTLV infection. *Int J Cancer* 1984; 33: 13-17.

189. Martin MP, Biggar RJ, Hamlin Green G, Staal S, Mann DL. Large granular lymphocytosis in a patient infected with HTLV-II. *AIDS Res Hum Retroviruses* 1993; 9: 715-719.
190. Maruyama K, Fukushima T, Kawamura K, Mochizuki S. Chromosome and gene rearrangements in immortalized human lymphocytes infected with human T-cell lymphotropic virus type 1. *Cancer Res* 1990; 50: 5697-5702.
191. Matsumoto M, Nomuta K, Matsumoto T, *et al.* Adult T-cell leukemia-lymphoma in Kagoshima District, South-western Japan: Clinical and haematological characteristics. *Jpn J Clin Oncol* 1979; 9: 325 – 336.
192. Matsuoka M, Hagiya M, Hattori T, *et al.* Gene rearrangements of T-cell receptor b and c chains in HTLV-I infected primary neoplastic T-cells. *Leukaemia* 1988; 2:84-90.
193. Matsuzaki, H, Asou N, Kawaguchi Y, *et al.* HTLV-1 associated with small cell lung cancer. *Cancer* 1990; 66:1763-1768.
194. Menitove JE. Blood donor HTLV-1 antibody testing. *Transfusion* 1989; 29:1-2
195. Merino F, Rubert-Guroff M, Clark J, Biondo-Bracho M, Blattner WA, Gallo RC. Natural antibodies to human T-cell leukaemia lymphoma virus in healthy Venezuelan populations. *Int J Cancer* 1984; 34:501-506.
196. Meselle SY, Shao JF, Haukenes G. Prevalence of antibodies to human T-lymphotropic virus type 1 in an HIV-endemic region of Africa. *E Afr Med J* 1990; 67, 461 – 465.
197. Miedema F, Tepstra FG, Smit JW, *et al.* Functional properties of neoplastic T- cell in adult T-cell lymphoma leukemia patients from the Caribbean. *Blood* 1984; 63:477-481.
198. Miller DH, Newton MR, Rudge P, Cruickshank K. Magnetic resonance imaging in HTLV-1 antibody positive patients. *Lancet* 1987; 2:514.
199. Minamoto GY, Gold JWM, Scheinberg DA, *et al.* Infection with human T-cell leukemia virus type-I in patients with leukemia. *N. Engl J Med* 1988; 318:219-222.
200. Miyamoto K, Sato J, Kitajima K-I, *et al.* Adult T-cell leukaemia. *Cancer* 1983; 52: 472-473.

201. Miyamoto K, Tomita N, Ishii A, *et al.* Transformation of ATLA-negative leucocytes by blood components from anti-ATLA-positive donors invitro. *Int J Cancer* 1984; 33:721-725.
202. Miwa M, Shimotohno K, Hoshimo M, Fujino M, Sugimura T. Detection of pX antigen in human T-cell leukemia virus (HTLV) – infected cells by using antibody against peptide deduced from sequences of X – IV DNA of HTLV-I and XcDNA of HTLV-II proviruses. *Gann* 1984; 75: 752 – 755.
203. Miwa M, Shimotohno K, Kitamura T, *et al.* HTLV and ATL. In: Gallo RC, Haseltine W, Klein G, zur Hausen H, eds. *Viruses and human cancer, UCLA symposia on Molecular and Cellular Biology, Park City, Utah*. Vol. 43. New York; Alan R, Liss; 1987; 131 – 140.
204. Miyamoto K, Tomita N, Ishii A, *et al.* Transformation of ATL A-negative leukocytes by blood components from anti-ATL-positive donors in vitro. *Int J Cancer* 1984; 33:721-725.
205. Miyoshi I, Taguchi H, Fujishita M, *et al.* Transformation of monkey lymphocytes with adult T-cell leukaemia virus. *Lancet* 1982; 1:1016.
206. Miyoshi I, Yashimoto S, Kubonishi J, *et al.* Transformation of normal human cord lymphocytes by co-cultivation with a lethally irradiated human T-cell line carrying type C particles. *Gann* 1981; 72: 997 – 998.
207. Miyoshi I, Yoshimoto S, Taguchi II, *et al.* Transformation of rabbit lymphocytes with T-cell leukemia virus. *Gann* 1983; 74: 1 –4.
208. Mochizuki M, Yamaguchi K, Takatsuki K, *et al.* HTLV-1 uveitis: a distinct clinical entity caused by HTLV-1. *Jpn J Cancer Res* 1992; 83:236-239.
209. Mochizuki M, Yamaguchi K, Takatsuki K, Wanatabe T, Mori S, Tajima K. HTLV-1 and uveitis. *Lancet* 1992; 339: 1110.
210. Mollison PL, Engelfriet CP, Marcela C. Blood Transfusion in Clinical Medicine. *Blackwell Scientific Publications, Oxford, U.K. pp 1-56.*

211. Montagnier L, Gruet J, Chamaret S. Adaptation of lymphadenopathy associated virus (LAV) to replication in EBV-transformed B lymphoblastoid cell lines. *Science* 1984; 225:65-66.
212. Mori N, Yamashita U, Tanaka Y, *et al.* Immuno suppressive factor produced by a B-cell line derived from an adult T-cell leukemia patient. *Int J Hematol* 1992; 56:29-39.
213. Morimoto C, Matsuyama T, Oshige C, *et al.* Functional and phenotypic studies of Japanese adult T-cell leukemia cells. *J Clin Invest* 1985; 75:836-843.
214. Msamanga, G. I. and Fawzi, W.W. (1997): The double burden of HIV infection and tuberculosis in sub-Saharan Africa. *N. Engl. J Med.*, 337; 849-851.
215. Murai K, Tachibana N, Shioiri S, *et al.* Suppression of delayed type hypersensitivity to PPD and PHA in elderly HTLV-1 carriers. *J Acquir Immune Defic Syndr* 1990; 3:1006-1009.
216. Murphy EL, Blatter WA, (1988) : HTLV-1 associated leukaemia: a model for chronic retroviral disease. *Ann. Neurol.*, 23S, S174-S180.
217. Nagashima K, Yoshida M, Seiki M. A single species of pX mRNA of HTLV-I encodes trans-activator p40x and two other phosphoproteins. *J Virol* 1986; 60: 394 -399.
218. Nagasato K, Nakamura T, Shirabe S, *et al.* Presence of serum anti-human T-lymphotropic virus 1 (HTLV-1) IgM antibodies means persistent active replication of HTLV-1 in HTLV-1-associated myelopathy. *J Neurol Sci* 199; 103:203-208.
219. Nagy K, Clapham P, Cheingsong-Popov R, Weiss RA. HTLV-I: induction of syncytia and inhibition by patients' sera. *Int. J Cancer* 1983; 32: 321 – 328.
220. Nagy K, Weiss RA, Clapham P, Cheingsong-Popov R. Biological properties of human T-cell leukaemia virus envelope antigens. In: Gallo RC, Essex M, Gross L, eds. *Human T-cell leukaemia viruses*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1984:121-131.
221. Nakada K, Kohakura M, Komoda H, Hinuma Y. High incidence of HTLV antibody in carriers of *Strongyloides stercoralis*. *Lancet* 198<sup>+</sup>; 1:833.

222. Nakano S, Ando Y, Ichijo M, *et al.* Search for possible routes of vertical and horizontal transmission of adult T-cell leukaemia virus. *Gann* 1984; 75:1044-1045.
223. Nakao K, Ohba N, Matsumoto M. Noninfectious anterior uveitis in patients infected with human T-lymphotropic virus type 1. *Jpn J Ophthalmol* 1989; 33: 472- 481.
224. Nam SH, Copeland Td, Hatamaka, Oroszlan S. Characterisation of ribosomal frame-shifting for expression of pol gene products of HTLV-I. *J. Virol* 1993; 67: 196 – 203.
225. Nam SH, Kidokoro M, Shida H, Hatamaka M. Processing of gag precursor polyprotein of HTLV-I by virus-encoded protease. *J Virol* 1988; 62: 3718 – 3728.
226. Neequaye AR, Mingle AA, Neequaye JE, Agadzi VK, Netty V, Osei-Kwasi M, Hayami M, Ishikawa K, Ankra-Badu G, Bentsi C, Asamoah-Adu S, Aggrey SE, Ampofo W, Brandful JA, Grant F, Biggar RJ. (1986): A report on human immunodeficiency virus (HIV) infection in Ghana up to December 1986. *Ghana Med. J.* 21, 7-12
227. Newton M, Miller D, Rudge P, *et al.* Antibody to human T-lymphotropic virus type 1 in West-Indian-born UK residents with spastic paraparesis. *Lancet* 1987; 1:415.
228. Newton RC, Limpuangthip P, Greenberg S, Gam A, Neva FA. Strongyloides stercoralis hyperinfection in a carrier of HTLV-1 virus with evidence of selective immunosuppression. *Am J Med* 1992;92: 202-208.
229. Nishioka K, Makajima T, Hasunuma T, Sato K. Rheumatic manifestation of human leukemia virus infection. *Rheum Dis Clin North Am* 1993; 19:489-503.
230. Nyborg JK, Dynan WS. Interaction of cellular proteins with the HTLV-I transcriptional control region. Purification of cellular proteins that bind the 21-base pair repeat elements. *J Biol Chem* 1990; 265: 8230 – 8236
231. Nyborg JK, Matthews MAH, Yeel J, *et al.* Interaction of host cell proteins with the HTLV-I transcriptional control region. II. A comprehensive map of protein-binding sites facilitates construction of a simple climatic promoter responsive to the viral tax 2 gene product. *J Biol Chem* 1990; 265: 8237 – 8242.

232. O'Brien SJ, Lampert JA, Catovsky D. The histopathology of adult T-cell lymphoma leukemia in blacks from Caribbean. *Histopathology* 1983; 7:349-364.
233. O'Doherty MJ, Van De Pette JE, Nunar TO, Croll DN. Recurrent strongyloides stercoralis infection in a patient with T-cell lymphoma-leukemia. *Lancet* 1984; 1:858.
234. Ohama E, Horikawa Y, Shimizu T, *et al.* Demyelination and remyelination in spinal cord lesions of human lymphotropic virus type-1-associated myelopathy. *Acta Neuropathol* 1990 ; 81:78-83.
235. Ohtani K, Nakamura M, Saito S, *et al.* Identification of two distinct elements in the long terminal repeat of HTLV-1 responsible for maximum gene expression. *EMBO J* 1987; 6:389-395.
236. Okachi K, Sato H, Hiruma YA. A retrospective study on transmission of Adult T-cell leukemia virus by blood transfusion: seroconversion in recipients. *Vox Sang* 1984; 46: 245 – 253.
237. Okamura T, Shibuya T, Harada M, Niho Y. Successful treatment of chronic adult T-cell leukemia with Ubenimex. *Acta Haematol* 1992; 87:94-97.
238. Okochi K, Sato H, Hinuma Y. A retrospective study on transmission of adult-T-cell leukemia virus by blood transfusion; seroconversion in recipients. *Vox Sang* 1984; 46:245-253.
239. Okochi K, Sato H. Transmission of ATL (HTLV-I) through blood transfusion. In: Miwa M, ed. *Retroviruses in Human lymphoma leukemia*. Utrecht: Japanese Science Society Press; 1985:129-135.
240. Okpara RA, Williams E, Schneider J, Wendler I, Hunsmann G. Antibodies to human T-cell leukemia virus types- I and-III in blood donors from Calabar, Nigeria. *Ann Intern Med* 1986; 104:132.

241. Oroszlan S, Copeland TD, Kalyanaraman VS, Sarngadharan MG, Gallo RC. Complete amino acid sequence of human T-cell leukemia virus structural protein, p15. *FEBS Lett* 1983; 162: 390 – 395.
242. Oroszlan S, Copeland TD, Kalyanaraman VS, Sarngadharan MG, Schultz AM, Gallo RC. Chemical analyses of human T-cell leukemia virus structural proteins. In: Gallo RC, Essex ME, Gross L, eds: Human T-cell leukemia lymphoma viruses. *Coldspring Harbor, NY; Coldspring Harbor Laboratory; 1984: 101 – 110.*
243. Osame M, Igata A, Matsumoto M, Kohka M, Usuku K, Izumo S. HTLV-1 associated myelopathy (HAM) treatment trials, retrospective survey and clinical and laboratory findings. *Hematol Res Commun* 1989; 3:271-284.
244. Osame M, Igata A, Matsumoto M, Usuku K, Izumo S, Kosaka K. HTLV-1 associated myelopathy: a report of 85 cases. *Ann Neurol* 1987; 2:116.
245. Osame M, Izumo S, Igata A, *et al.* Blood transfusion with HTLV-1 associated myelopathy. *Lancet* 1982; 2: 104-105.
246. Osame M, Matsumoto M, Usuku K, *et al.* Chronic progressive myelopathy associated with elevated antibodies to human T-lymphotropic virus type 1 and adult T-cell leukaemia-like cell. *Ann Neurol* 1987; 21:117-122.
247. Osame M, Usuku K, Izumo S, *et al.* HTLV-1 associated myelopathy a new clinical entity. *Lancet* 1986; 1:1031-1032.
248. Page JB, Lai S, Chitwood DD, Klimas NG, Smith PC, Fletcher MA. HTLV-I/II seropositivity and death from AIDS among HTLV-I seropositive intravenous drug users. *Lancet* 1990; 335:1439-1441.
249. Paine E, Garcia J, Philpott TC, Shaw G, Rotner L. Limited sequence variation in human T-lymphotropic virus type I isolates from North American and African patients. *Virology* 1991; 2:111 – 123.

250. Pallela FJ Jr., Delaney K.M, Moorman AC, Loveless MD, Fuhrer J. and Satter G A. (1998): Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N. Engl. J Med.*, 338:853-860.
251. Pape JW, Liautaud B, Thomas F: (1983): Characteristics of the acquired immunodeficiency syndrome (AIDS) in Haiti. *N. Engl. Med.*, 309, 945-950.
252. Parkman PD. HTLV-I antibody testing: *FDA CBER Memo* Nov,29 1988.
253. Parker CE, Daenke S, Nightingale S, Bangham C. M. Activated HTLV-1-specific cytotoxic T-lymphocytes are found in healthy seropositives as well as in patients with tropical spastic paraparesis. *Virology* 1992; 188: 628-636.
254. Paskallis H, Felber BK, Pavlakis GN. Cis-acting sequences responsible for the transcriptional activation of HTLV-I constitute a conditional enhancer. *Proc Natl. Acad Sci. USA* 1986; 83: 6558 – 6562.
255. Pennington J, Taylor GP, Sutherland J, Davis RE, Seghatchian J, Allian JP, Williamson LM. Persistence of HTLV-I in blood components after leucocyte depletion. *Blood* 2002; 100:677-681.
256. Pique C, Tursz T, Dokhela MC. Mutations introduced along the HTLV-1 envelope gene in a non-functional protein: a basis for conservation? *EMBO J* 1990; 9: 4243-4248.
257. Poesz BJ, Ruscetti FW, Gasdar AF, *et al.* Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl. Acad Sci USA.* 1980; 77: 7415 – 7419.
258. Popovic M, Lange-Wilntzin G, Sarin PS, Mann D, Gallo RC. Transformation of human of human umbilical cord blood T-cells by human T-cell leukemia lymphoma virus. *Proc Natl Acad Sci USA* 1983; 80: 5402 – 5406.
259. Popovic M, Reitz MS Jr., Sarngadharan MG, *et al.* The virus of Japanese Adult T-cell leukemia is a member of the human T-cell leukemia virus group. *Nature* 1982; 300: 63 – 66.

260. Popovic M, Sarngadharan, MG, Read E, Gallo RC. Detection, isolation and continuous production of cytopathic virus (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 1984; 224: 497-500.
261. Popovic M., Sarin P S, and Robert-Guroff M. (1983): Isolation and transmission of human retrovirus (HTLV). *Science* 1983; 219, 856-859.
262. Rahlenbeck SI, Yohhanes G, Molla K, Reifen R, Assefa A. Infection with HIV, syphilis and HBV in Ethiopia: a survey in blood donors. *Int J STD/AIDS* 1997; 8, 261 – 264.
263. Ratner L, Joseph SF, Starch B, *et al.* Nucleotide sequence analysis of a variant human T-cell leukemia virus (HTLV-Ib) provirus with a deletion in pX-1. *J Virol* 1985; 54: 781 – 790.
264. Reisz MS Jr, Popovic M, Haynes BF, Clark SC, Gallo RC. Relatedness by nucleic acid hybridisation of new isolates of human T-cell leukaemia-lymphoma virus (HTLV) and demonstration of provirus in uncultured leukemic blood cell<sup>1</sup> *Virology*. 1983; 126:688-692.
265. Rho HM, Poiesz BJ, Ruscetti FW, Gallo RC. Characterization of the reverse transcriptase from a new retrovirus (HTLV) produced by a human cutaneous T-cell lymphoma cell line. *Virology* 1981; 112: 355 – 360.
266. Richardson JII, Edwards AJ, Cruickshank JK, Rudge P, Dalgeish AG. In vivo cellular tropism of HTLV-I. *J Virol* 1990; 64: 5682 -5687.
267. Robert-Guroff M, Fahey KA, Maedo M, Nakao Y, Ito Y, Gallo RC. Identification of HTLV p19 specific natural human antibodies by competition with monoclonal antibody. *Virology* 1982; 122: 297-305.
268. Robert-Guroff M, Clark J, Lanier AP, *et al.* Prevalence of HTLV-1 in arctic regions. *Int J Cancer* 1985; 36:651-665.
269. Robert-Guroff M, Schuphach DW, Blayney DW, Kalyanaraman VS, Merino F, Gallo RC. Seroepidemiologic studies on human T-cell leukemia lymphoma virus, type 1. In Gallo RC Essex ME, Gross L, eds. *Human T-cell leukemia lymphoma virus*. Cold spring Harbor, NY: Cold Spring Harbor laboratory; 1984; 285-295.

270. Robert-Guroff, M., Kalyanaraman, V.S. and Blattner W. A. (1983): Evidence for human T-cell lymphoma leukaemia infection of family members of human T-cell lymphoma leukaemia virus positive T-cell lymphoma leukaemia patient. *J. Exp. Med.* 187,248-258.
271. Robert-Guroff M, Weiss SH, Giron JA, *et al.* Prevalence of antibodies to HTLV-1, II and – III in intravenous drug abusers from an AIDS endemic region. *JAMA* 1986; 255:3133-3137.
272. Robinson RD, Murphy EL, Wilks RJ, Neva FA, Terry SI, Hanchard B, Figuevos JP, Blattner WA. Gastrointestinal parasitic infection in healthy Jamaican carriers of HTLV-1. *J Trop Med Hyg* 1991; 94:411-415.
273. Rodgers-Johnson P, Garruto R, Gajdusek DC, Mora C, Zaminovic V, St. C, Morgan O. Tropical spastic paraparesis: evidence for the etiological role of human T-cell lymphotropic virus type 1 in endemic foci in Jamaica and Columbia. *Ann Neurol* 1987; 22:116-117.
274. Rosen CA, Park R, Sodroski JG, Haseltine WA. Multiple sequence elements are required for regulation of human T-cell leukemia virus required for regulation of human T-cell leukemia virus gene expression. *Proc. Natl Acad Sci USA* 1987; 81: 4919 – 4923.
275. Rosenblatt JD, Chen ISY, Wachsman W. Infection with HTLV-1 and HTLV-II- evolving concepts. *Semin Hematol* 1988;25: 230-246.
276. Rosenblatt JD, Giorgi JV, Golde DW, *et al.* Integrated HTLV-II genome in CD 8-T-cells from a patient with ‘atypical’ hairy-cell leukaemia: evidence for distinct T and B-cell lymphoproliferative disorders. *Blood* 1988; 71: 363 – 366
277. Rosenblatt JD, Golde DW, Wachsman W, *et al.* A second HTLV-II isolate associated with atypical hairy-cell leukaemia. *N. Engl J Med* 1986; 315: 372 – 375.
278. Rosenblatt JD, Plaeger-Marshall S, Giorgi JV, *et al.* A clinical, haematologic, and immunologic analysis of 21 HTLV-II-infected intravenous drug users. *Blood* 1990; 76: 409-417.

279. Ruscetti FW, Robert Gureff, Ceccherinin Nelli L, *et al.* Persistent in vitro infection by human T-cell leukaemia-lymphoma virus (HTLV) of normal human T-lymphocytes from blood relations of patients with HTLV-associated mature T-cell neoplasms. *Int J Cancer* 1983; 31: 17 – 180.
280. Saito S, Furuki K, Ando Y, *et al.* Identification of HTLV-I sequence in cord blood mononuclear cells of neonates born to HTLV-I antigen/antibody-positive mothers by polymerase chain reaction. *Jpn J Cancer Res* 1990; 81:890-895.
281. Saji F, Ohashi K, Tokugawa Y, Kamiura S, Azuma C, Tanizawa O. Perinatal infection of human T-lymphotropic virus type I, the etiologic virus of adult T-cell leukemia/lymphoma. DNA amplification of specific human T-lymphotropic virus type I sequence. *Cancer* 1990; 66:1933-1937.
282. Sanada I, Tanaka R, Kumaga E, *et al.* Chromosomal aberrations in adult-T cell leukaemia; relationship to the clinical severity. *Blood* 1985; 65: 649-654.
283. Sato H, Okochi K. Transmission of human T-cell leukaemia virus (HTLV-1) by blood transfusion: demonstration of proviral DNA in recipient's lymphocytes. *Int J Cancer* 1986; 37:395-400.
284. Sato K, Maruyama I, Maruyama Y, *et al.* Arthritis in patients with human T lymphotropic virus type 1: clinical and immunopathologic features. *Arthritis Rheum* 1991; 34: 714-721.
285. Sarkodie F, Adarkwa M, Adu-Sarkodie Y, Candotti D, Acheampong JW, Allain JP. Screening for viral markers in volunteer and replacement blood donors in West Africa. *Vox Sang* 2001; 80, 142 – 147.
286. Sarngadharar, M. G. Popovic, M, Bruch, L., Schupbach, J. and Gall, R. C. (1984): Antibodies reactive with T-cell lymphotropic retrovirus (HTLV-II) in the serum of patients with AIDS. *Science* 224, 506-508.

287. Saxinger C, Gallo RC. Methods in laboratory investigation of the indirect enzyme-linked immunosorbent assay microtest to the detection and surveillance of human T-cell leukaemia lymphoma virus. *Lab Invest.* 1983; 49: 371-377.
288. Saxinger WC, Lange-Wantzin G, and Thomsen K, *et al.* Human T-cell leukaemia virus: a diverse family of related exogenous retroviruses of humans and old world primates. In: Gallo RC, ed. *Human T-cell leukaemia lymphoma virus.* Cold Spring Harbor, NY: Cold Spring Harbour Laboratory; 1984:323-330.
289. Saxinger W, Blattner WA, Levine PH, Clark J, Biggar R, Hoh M, Moghissi J, Jacobs P, Wilson L, Jacobson R, Crookes R, Strong M, Ansari AA, Dean AG, Nkrumah FK, Mourali N, Gallo RC. Human T-cell leukaemia virus (HTLV-1) antibodies in Africa. *Science* 1984; 225:1473-1476.
290. Saxinger WC, Wantzin GL, Thomsen K, Hoh M, Gallo RC. Occurrence of HTLV-1 antibodies in Danish patients with cutaneous T-cell lymphoma. *Scand J Haematol* 1985; 31:455-462.
291. Saxon A, M. Steven RH, Golde DW. T-lymphocyte variant of hairy-cell leukaemia. *Ann Intern Med.* 1978; 88:323 – 326.
292. Saxon A, M. Steven RH, Quan S.G., Golde DW. Immunologic characterization of hairy-cell leukaemia in continuous culture. *J Immunol* 1978; 120: 777 – 782.
293. Schneider J, Yamamoto N, Hinuma Y, Hunsmann G. Precursor polypeptides of adult-T cell leukaemia virus; detection and antisera against isolated polypeptides gp68, p24 and p29. *J Gen Virol* 1984; 65: 2249-2258.
294. Schneider J, Yamamoto N, Hinuma Y, Hunsmann G. Sera from adult T-cell leukaemia patients react with envelope and core polypeptides of adult T-cell leukaemia virus. *Virology* 1984; 132:1– 11.

295. Schulz TF, Calabro ML, Hoad JG *et al.* HTLV-I envelope sequences from Brazil, the Caribbean, and Romania: clustering of sequences according to geographic origin and variability in an antibody epitope. *Virology* 1991; 4: 3 – 491.
296. Schupbach J, Kalyanarama VS, Sarnagadharana MG, Blattner WA, Gallo RC. Antibodies against three purified proteins of the human type C retrovirus, human T-cell leukaemia-lymphoma virus, in adult T-cell leukaemia –lymphoma patients and healthy blacks from Caribbean. *Cancer Res* 1983; 43: 886-891.
297. Schupbach J, Kalyanarama VS, Sarnagadharan MG, Nakao Y, Ito Y, Gallo RC. Antibodies against three purified structural proteins on human type C retroviruses, HTLV, in Japanese adult T-cell leukaemia patients, healthy family members, and unrelated normal. *Int J Cancer* 1983; 32:583-590.
298. Seiki M, Eddy R, Shows TB, Yoshida M. Non-specific integration of the HTLV provirus genome into adult T-cell leukaemia cells. *Nature* 1984; 309:640-642.
299. Seiki M, Hatton S, Hirayama Y, Yoshida M. Human adult T-cell leukaemia virus: molecular cloning of the provirus DNA and unique terminal structure. *Proc Natl Acad Sci USA* 1982; 79: 6899 – 6902.
300. Seiki M, Hikikoshi A, Taniguchi T, Yoshida M. Expression of the pX gene of HTLV –I: general splicing mechanism in the HTLV family. *Science* 1985; 228: 1532 – 1535
301. Seto A, Isono T, Ogawa K. Infection of inbred rabbits with cell-free HTLV-I. *Leuk Res* 1991; 15: 105 – 110.
302. Sherman MP, Saksena NK, Dube DK, Yanagihara R, Poiesz BJ. Evolutionary insights on the origin of human T-cell lymphoma/leukemia virus type I (HTLV-I) derived from sequence analysis of a new HTLV-I variant from Fapua New Guinea. *J Virol* 1992; 66: 2556 – 2563.
303. Shibata A, Aoyagi Y, Aoki S. Adult T-cell leukaemia observed at Niigata University Hospital. *Jpn J Clin Oncol* 1983; 13:657-666.

304. Shima H, Takano M, Shimotohno K, Miwa M. Identification of p26xb and p24xb of HTLV-II. *FEBS Lett* 1986; 209:289 – 294.
305. Shimada K, Koh CS, Yanagisawa N, Tsukuda N, Osame M. Anti-lymphocyte antibodies and circulating immune complexes in the sera of patients with myelopathy associated with human T lymphotropic virus type-1. *J Neuroimmunol* 1993; 42: 161-166.
306. Shimotohno K, Takano M, Trunchi T, Miwa M. Requirement of multiple copies of a 21-nucleotide sequence in the U3 regions of HTLV-I and type II long terminal repeats for trans-activation of transcription. *Proc Natl Acad Sci USA* 1986; 83: 81112 – 8116.
307. Shimoyama M, Minato K, Tobinai K, *et al.* Atypical adult T-cell leukemia-lymphoma: diverse clinical manifestations of adult T-cell leukemia-lymphoma. *Jpn J Clinical Oncol* 1983; 13: 165 – 187.
308. Shimoyama M, Ota K, Kikuchi M, *et al.* Chemotherapeutic results and prognostic factors of patients with advanced non-Hodgkin's lymphoma treated with VEPA or EFPA-M. *J Clin Oncol* 1988; 6: 128-141.
309. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia- lymphoma. A report from the lymphoma study group (1984 – 1987). *Br J Haematology* 1991; 79: 437.
310. Slamon DJ, Boyle WJ, Keith DE, Press MF, Golde DW, Souza LM. Subnuclear localization of the trans-acting protein of HTLV-I. *J Virol* 1988; 62: 680 – 686.
311. Slamon DJ, Shimotohno K, Cinc MJ, Golde DW, Chen ISY. Identification of the putative transforming protein of the human T-cell leukaemia viruses HTLV-1 and HTLV-II. *Science* 1984; 226: 61 – 65.
312. Smith MR, Greene WC. Characterization of a novel nuclear localization signal in the HTLV-I tax trans-activator protein. *Virology* 1997; 187: 316 – 320.

313. Smith TF. Structure, classification and replication of viruses In: Insalaco SJ, Mentove JE, eds. Transfusion transmitted viruses: epidemiology and pathology. *Arlington, VA: American Association of Blood Banks*, 1987:1-16.
314. Sodroski JG, Rosen CA, Haseltine WA. Trans-acting transcriptional activation of the long terminal repeat of human T lymphotropic virus in infected cells. *Science* 1984; 225: 381 – 385.
315. Sohn CC, Blayney DW, Misset JL, *et al.* Leukopenic chronic T-cell leukemia mimicking hairy cell leukemia: association with human retrovirus. *Blood* 1986; 67:949-956.
316. Sommerfelt MA, Weiss RA. Receptor interference groups of 20 retroviruses plating on human cell. *Virology* 1990; 176: 58 – 69.
317. Sommerfelt MA, Williams BP, Clapham PR, Solomon E, Goodfellow PN, Weiss RA. Human T-cell leukemia viruses use a receptor determined by human chromosome 17. *Science* 1988; 142: 1557 – 1559.
318. Starkebaum G, Kalyanaraman VS, Kidd PG, *et al.* Serum reactivity to human T- cell leukemia lymphoma virus type 1 proteins in patients' with large granular lymphocytic leukemia. *Lancet* 1987; 1:596-598.
319. Su IJ, Chan HL, Kao TT, *et al.* Adult T-cell leukaemia lymphoma in Taiwan. *Cancer* 1985; 56: 2217-2220.
320. Sugamura K, Fujii M, Kannagi M, Sakitani M, Takeuchi M, Hinuma Y. Cell surface phenotypes and expression of viral antigens of various human cell lines carrying adult T-cell leukemia virus. *Int. J Cancer* 1984; 34:221-228.
321. Sullivan MT, Williams AE, Fang CT, *et al.* Transmission of human T-lymphotropic virus type-I and-II by blood transfusion: a retrospective study of recipients of blood components. *Arch Intern Med* 1991; 151:2043-2048.

322. Taguchi H, Sawada T, Fujishita M, Morimoto T, Naya K, Miyoshi I. Enzyme-linked immunosorbent assay of antibodies to adult T-cell leukaemia-associated antigens. *Gann* 1983; 74: 185-187.
323. Taguchi H, Kobayashi M, Miyoshi I. Immuno-suppression by HTLV-1 infection (I). *Lancet* 1991; 337:309.
324. Taguchi H, Sonobe H, Yamato H, *et al.* Central nervous system involvement of adult T-cell leukaemia- lymphoma with multinucleated giant cells in the brain, skin, and kidney. *Cancer* 1993; 71: 133-137.
325. Tajima K. The 4<sup>th</sup> nation-wide study of adult T-cell leukaemia/lymphoma (ATL) in Japan: estimates of risk of ATL and geographical and clinical features. *Int J Cancer* 1990; 45: 237-243.
326. Tajima K, Tominaga S, Suchi T, *et al.* Epidemiological analysis of the distribution of antibody to adult T-cell leukemia-virus associated antigen: possible horizontal transmission of adult T-cell leukemia virus. *Gann* 1982; 73:893-901.
327. Tajima K, Tominaga S, Kuroishi T, Shimizu H, Suchi T. Geographical features and epidemiological approach to endemic T-cell leukaemia lymphoma in Japan. *Jpn J Clin Oncol* 1979; 9:495-504.
328. Tajima K, Tominaga S, Suchi T. Clino-epidemiological analysis of adult T-cell leukaemia. *Gann Monogr Cancer Res* 1982; 28:197-210.
329. Takatsuki K, Uchiyama T, Ueshima Y, Hattori T. Further Clinical observations and cytogenetic and functional studies of leukemic cells. *Jpn J Clin Oncol* 1979; 9: 312 – 324
330. Takatsuki K, Uchiyama T, Ueshima Y, *et al.* Adult T-cell leukemia: proposal as a new disease and cytogenetic, phenotypic and functional studies of leukemic cells. *Gann Monogr Cancer Res.* 1982; 28:13-21.
331. Takatsuki K, Uchiyama T, Ueshima Y, Hattori T. Further clinical observations and cytogenetic and functional studies of leukemic cells. *Jpn J Chin Oncol* 1979; 9:312-324.

332. Takenaga T, Nakamine P, Oshino I, *et al.* Serologic and epidemiologic studies on adult T-cell leukaemia (ATL): special reference to a comparison between patients with and without antibody to ATL-associated antigen. *Jpn J Clin Oncol* 1983; 13:257-268.
333. Takeuchi K, Kobayashi N, Nam SH, Yamamoto H, Hatena M. Molecular cloning of cDNA encoding gp68 of adult T-cell leukaemia associated antigen: evidence for expression of pXIV region of human T-cell leukaemia virus. *J Gen Virol* 1985; 66:1825-1829.
334. Takeuchi Y, Simpson G, Vile RG, Weiss RA, Collins MK I. Retroviral pseudotypes produced by rescue of a Moloney murine leukemia virus vector by C-type; but not D-type retroviruses. *Virology* 1992; 186: 792 – 794.
335. Tateno M, Kondo N, Itoh T, Chubachoi T, Togashi T, Yoshiki T. Rat lymphoid lines with human T-cell leukemia virus production. Biological and serological characterization. *J Exp med* 1984; 169: 1105 – 1116.
336. Tedder RS, Shanson DC, Jeffries DJ, *et al.* Low prevalence in the U.K. of HTLV-1 and HTLV-II infection in subjects with AIDS extended lymphadenopathy and at risk of AIDS. *Lancet* 1984; 2: 125-128
337. The G, Gessain A, Gazzalo A, Rober-guroff M, Najberg G, Calender A, Peti M, Brubaker G, Bensliman A, Fabry F, Strobel M, Robin Y, Fortune R. Comparative seroepidemiology of HTLV-I and HTLV-III in the French West Indies and some African countries. *Cancer Res* 1985; 45, 4633S – 4636S.
338. Tokunaga M, Hasui K, Sato E. Ultrastructure of adult T-cell lymphoma. *Gann* 1982; 73: 748-757.
339. Tournier-Lasserve E, Gout O, Gassain A, *et al.* HTLV-1, brain abnormalities on magnetic resonance imaging, and relation with multiple sclerosis. *Lancet* 1987; 2:49-50.
340. Tsuji Y, Doi H, Yamabe T, Ishimaru T, Miyamoto T, Hino S. Prevention of mother-to-child transmission of human T-lymphotropic virus type-I. *Pediatrics* 1990; 86:11-17.

341. Tsujimoto H, Terunchi T, Imamura J, Shimotohno K, Miyoshi I, Miwa M. Nucleotide sequence analysis of a provirus derived from HTLV-I-associated myelopathy (HAM). *Mol Biol Med* 1988; 5: 29 – 42.
342. Uchiyama T, Nelson DL, Fleisher TA, Waldman TA. A monoclonal antibody (anti-tac) reactive with activated and functionally mature human T-cell. 11: Expression of tac antigen on activated cytotoxic killer cells, suppressor cells, and on one or two types of helper T-cells. *J Immunol* 1981; 126:1398-1403.
343. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: Clinical and hematologic features of 16 cases. *Blood* 1977; 50: 481 -492.
344. Ueda N, Iwata K, Tokuoka H, Akagi T, Ho J, Mizushima M. Adult T-cell leukemia with generalized cytomegalic inclusion disease and *Pneumocystis carinii* pneumonia. *Acta Pathol Jpn* 1979; 29:221-232.
345. Ueshima Y, Furuhashi S, Hattori S, Uchiyama T, Takatsuki K, Uchino H. Chromosome studies in adult T-cell leukemia in Japan significance of trisomy 7. *Blood* 1988; 58:420-425.
346. Usuku K, Nishizawa M, Osame M, Tabira T. Cytotoxic and suppressor activities in patients with HTLV-1- associated myelopathy. *J Neuroimmunol* 1991; 33: 199-205.
347. VandenHock JAR, Al EJM, Hunsman JG, Goudsmit J, Coutinho RA. Low prevalence of human T-cell leukaemia virus type- I, and -II, infection among drug users in Amsterdam, The Netherlands. *J Med Virol* 1991; 34:100-103.
348. Verdier M, Denis F, Sangare A, Barin F, Gershy-Damet G, Rey JL, Benoit S, Leonard G, Mounier M, Hugon J. Prevalence of antibody to human T cell leukaemia virus type 1 in populations of Ivory Coast. *J Infect Dis* 1989; 160, 363-370, 1989.
349. Vernant JC, Maurs L, Gessain A, *et al*. Endemic tropical spastic paraparesis, associated with human T-lymphotropic virus type 1: a clinical and sero-epidemiological study of 25 cases. *Ann Neurol* 1987; 2:123-130.

350. Vieira J, Frank E, Spira TJ, Landesman SH. (1983): Opportunistic infections in previously healthy Haitian immigrants. *N. Engl. J. Med.*,308, 125-129.
351. Vos J. Guidelines for appropriate prescribing of blood transfusions. *Post Grad Doct* 1998; 20, 36-38.
352. Vyth-Dreese FA, de Vries H. Human T-cell leukaemia patient originating from Surinam. *Lancet* 1982; 2: 993.
353. Vyth-Dreese FA, de Vries H. Phenotypic and functional characterization of HTLV positive neoplastic T-cell Cultured with interleukin-2. 1. Retention of morphology, phenotype and selective cytotoxic properties in longterm culture. *Leuk Res.* 1982; 8:1-14.
354. Wachsman W, Golde DW, Temple PA, Orr EC, Clark SC, Chen ISY. HTLV x gene product: requirement for the env methionine initiation codon.. *Science* 1985; 228: 1534 – 1537
355. Wachsman W, Shimotohno K, Clark SC, Golde D V, Chen 154. Expression of the 3' terminal region of human T-cell leukemia viruses. *Science* 1984; 226: 177 – 179.
356. Watanabe T, Yamaguchi K, Takatsuki K, Osame M, Yoshida M. Constitutive expression of parathyroid hormone-related protein gene in human T-cell leukemia virus type 1 (HTLV-1) carriers and adult T-cell leukemia patients that can be trans-activated by HTLV-1 tax gene. *J Exp Med* 1990; 172:759-765.
357. Weiss R. Tissue specific transformation by human T-cell leukemia virus. *Nature* 1984; 310: 273 – 274.
358. Weiss RA, Cheingsong-Popov R, Clayden S, *et al.* Lack of HTLV-1 antibodies in Africans. *Nature* 1986; 319:794-795.
359. Weiss RA, Clapham P, Nagy K, Hoshino H. Envelope properties of human T-cell leukemia viruses. *Curr Top Microbiol Immunol* 1985; 115: 235 – 246.
360. Weiss SH, Saxinger WC, Ginzburg HM. (1987): Human T-cell lymphotropic virus type-1 and HIV prevalences among US drug abusers (DA). *Proc. Am. Soc. Clin. Oncol.*, 6, 4-8.

361. Whang-Peng J, Bunn PA, Knutsen T, *et al.* Cytogenetic studies in human T-cell lymphoma virus (HTLV)-positive leukaemia lymphoma in the United States. *J Natl Cancer Inst* 1985; 74:357-369.
362. WHO Weekly Epidemiological Record on HCV. 14, November 1997.
363. Wiktor SZ, Jacobson S, Weiss SH, *et al.* Spontaneous lymphocyte proliferation in HTLV-II infection. *Lancet* 1991; 337: 327.
364. Williams AE, Fang CT, Slamon DJ, *et al.* Seroprevalence and epidemiological correlates of HTLV-I infection in American blood donors. *Science* 1988; 240: 643 – 646.
365. Wong-Staal F, Gallo RC. Human T-lymphotropic retrovirus. *Nature* 1985; 317:395-403.
366. Yamada Y, Kamihira S, Amagasaki T, *et al.* Adult T-cell leukemia with atypical surface phenotypes: clinical correlation. *J Clin Oncol* 1985; 3:782-788.
367. Yamada Y. Phenotype and functional analysis of leukemia cells from 16 patients with adult T-cell leukemia lymphoma. *Blood* 1983; 61: 192 – 199.
368. Yamade I, Isono T, Ishiguro T, Yoshida Y. Comparative study of human and rabbit cell infection with cell-free HTLV-I. *J Med Virol* 1993; 39: 75 – 79.
369. Yamaguchi K, Yoshioka R, Kiyokawa T, Seiki M, Yoshida M, Takatsuki. Lymphoma type adult T-cell leukemia - a clinicopathologic study of HTLV related T-cell type malignant lymphoma. *Hematol Oncol* 1986; 4:59-65.
370. Yamaguchi K, Nishimura H, Kohrogi H, Jono M, Miyamoto Y, Takatsuki K. A proposal for smoldering adult T-cell leukemia: a clinico- pathologic study of five cases. *Blood* 1983; 62: 758 – 766.
371. Yamamoto N, Chosa T, Koyanagi Y, Tochikura T, Schneider J, Hinuma Y. Binding of adult T-cell leukaemia virus to various hematopoietic cells. *Cancer Lett* 1984; 10: 989-993.

372. Yamamoto N, Hayami M, Komuro A, *et al.* Experimental infection of cynomolgous monkeys with a human retrovirus, adult T-cell leukemia virus. *Med Microbiol* 1984; 173: 57 – 64.
373. Yamamoto N, Hinuma Y. Antigens in an adult T-cell leukaemia viral producer cell line; reactivity with human serum antibodies. *Int J Cancer* 1982; 30:289-293.
374. Yamamoto N, Matsumoto T, Koyanagi Y, Tanaka Y, Hinuma Y. Unique cell lines harbouring both Epstein-Barr virus and adult T-cell leukaemia virus, established from leukaemia patients. *Nature* 1982; 299: 367-369.
375. Yamamoto N, Okada M, Koyanagi Y, Kannagi M, Hinuma Y. Transformation of human leukocytes by co-cultivation with an adult T-cell leukemia virus producer cell line. *Science* 1982; 217: 737 – 739.
376. Yamamoto N, Schneider J, Hinuma Y, Hunsmann G. Adult T-cell leukaemia-associated antigen (ATLA): detection of a glycoprotein in cell- and virus-free supernatant. *ZNaturforsch (C)* 1982; 37c: 731-732.
377. Yamanouchi K, Kinoshita K, Moriuchi R, *et al.* Oral transmission of HTLV-I into a common marmoset (*Callithrix jacchus*) as an experimental model for milk-borne transmission. *Gann* 1985; 76:481-487.
378. Yoshida M, Hattori S, Seiki M. Molecular biology of human T-cell leukaemia virus associated with adult T-cell leukaemia. *Curr Top Microbiol Immunol* 1985; 115: 157-175.
379. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proct Ntl. Acad Sci USA* 1982; 79: 2031 – 2035.
380. Yoshida M, Seiki M, Yamaguchi K, Takatsuki K. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult-c ll leukaemia suggests causative role of human T-cell leukemia suggest causative role of human T-cell leukemia virus in the disease. *Proc Natt Acad Sci USA* 1984; 81:2534-2537.

381. Yoshikura H, Nishida J, Yoshida M, Kitamura Y, Takahu T, Ikeda S. Isolation of HTLV derived from Japanese adult T-cell leukaemia patients in human diploid fibroblast strain I MR 90 and the biological characters of the infected cells. *Int J Cancer* 1984; 33: 745 -749.
382. Yoshioka R, Yamaguchi K, Yoshinaga T, Takatsuki K. Pulmonary complications in patients with adult T-cell leukaemia. *Cancer* 1985; 55:2491-2494.
383. Zazopoulos E, Sodroski JG, Haseltine WA. P21 (rex) protein of HTLV-1. *J Acquir Immune Defic Syndr* 1990; 3:1135-1139.
384. Zucker-Franklin D, Hooper WC, Evatt BL, *et al.* Human lymphotropic retroviruses associated with fungoides; evidence that human T-cell lymphotropic virus type II (HTLV-II) as well as HTLV-1 may play a role in the disease. *Blood* 1992; 80: 1537-1545.



**APPENDIX**

**QUESTIONNAIRE**

1. Age of respondent Date of Birth
2. Educational level of respondent No schooling
- Primary School
- Middle School
- Secondary School
- Junior Sec. School
- Senior Sec. School
- University
- Other
3. Occupation of respondent: .....
- 4a. Marital status M/S
- 4b. Sexual preference Homosexual/Heterosexual
- 5a. If married, how many other wives/husbands do you have?
- 1
- 2
- 3
- 4
- None

5b. How many people have you been married to

- 1
- 2
- 3
- 4

6. Can you estimate the number male/

female sexual partners you have had? ...../Don't know

7. Have you been involved in any military

duties outside/Ghana?

Yes/No

8. If yes to question 7, were you involved in any

sexual activity during your military duties?

Yes/No

9. Did you use any contraceptive?

Yes/No

10. What contraceptive method do you use?

- Oral contraceptives
- Injectable
- Norplant
- Barrier method
- Condom

Female condom

Diaphragm

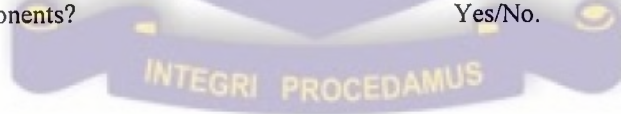
- None
- Other, please state

11. Have you ever donated blood? Yes/No

12. If yes to question 11, how many times?

- 1
- 2
- 3
- 4
- 5

13. Have you been transfused with blood or blood components? Yes/No.



14. If yes to question 13, how many times?

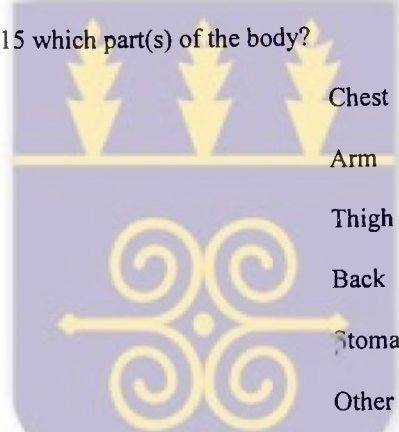
- 1
- 2
- 3
- 4

5

15. Do you have any tattoo marks on your body? Yes/No.

15.b When was the tattoo done?/How long have you had the tattoo?

16. If yes to question 15 which part(s) of the body?



Chest

Arm

Thigh

Back

Stomach

Other

17. Have you heard of human T-lymphotropic

virus type-1 disease ?

Yes/No