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

BY

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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.

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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. Wadhwani, 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= Human immunodeficiency virus
HTLV-1 = Human T-lymphotropic virus type-I
LAV= Lymphoadenopathy 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
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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 37th 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 ($X^2 = 1.72; \text{or } = 2.07; 95\% \text{ CI } = 0.16 - 1.46$). 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 37th 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, 37th 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 (13,362). 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 37th 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 serc-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 others parts of Africa; and that the sero-prevalence rate of antibodies to HTLV-1 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 HLTV-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-I 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,
infests 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 messengers 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) autocatalized 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 0.7864 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 Mg2+ 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 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-I 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 immuno-precipitate both proteins (fig 1).

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 m RNA'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
efficiency 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 fixed 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.
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-1 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 lymphoadenopathy that does not involve the mediastinum hepatosplenomegally, and skin lesions due to infiltration of leukemic cells. **Intestinal** pneumonitis, also caused by leukemic cell infiltration, is sometime present. Patients manifest abnormalities in certain serum chemistries, including elevation of lactate dehydrogenase (LDH) hyperbilirubinemia 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-1α 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 markers (39, 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-Hodgkin’s 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-Hodgkin’s 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 be 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 leukemias 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-1 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).
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


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,
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-I 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 transpental 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 derivates emphasizing 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-I. 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

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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 straightforward, 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 \textit{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 drawback. 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 37th 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 18.0g/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/cm3.

Seridia 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 2nd 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$_2$O$_2$ and 0.05% σ-phenylenediamine). The enzyme reaction was stopped, by adding 50μl 2M H$_2$SO$_4$, and the resulting colour was read on a Titertek plate reader at 92nm. 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.
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

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

### Table 3 – Occupation of Blood Donors

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Frequency</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accountant</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Baker</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>Caterer</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>Catechist</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Cleaner</td>
<td>14</td>
<td>1.1</td>
</tr>
<tr>
<td>Factory Hand</td>
<td>35</td>
<td>2.9</td>
</tr>
<tr>
<td>Hairdresser</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td>Housewife</td>
<td>12</td>
<td>1.0</td>
</tr>
<tr>
<td>Messenger</td>
<td>18</td>
<td>1.5</td>
</tr>
<tr>
<td>Nurse</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Seamstress</td>
<td>9</td>
<td>0.7</td>
</tr>
<tr>
<td>Salesgirl</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>Secretary</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>Social worker</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Student</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td>Tax officer</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Teacher</td>
<td>87</td>
<td>7.1</td>
</tr>
<tr>
<td>Telephonist</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Trader</td>
<td>701</td>
<td>57.2</td>
</tr>
<tr>
<td>Unemployed</td>
<td>300</td>
<td>24.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1225</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
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 MHBTC. Despite this limitation, the general observation is that blood donated at MHBTC, Accra, contain relatively high prevalence of antibodies to HTLV-I. 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


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-


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APPENDIX

QUESTIONNAIRE

1. Age of respondent

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 □
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