HUMAN T-CELL LYMPHOTROPIC VIRUSES

1. Exposure Data

1.1 Structure, taxonomy and biology

1.1.1 Structure

The structure of retroviruses is reviewed in the monograph on human immuno-deficiency viruses (HIV) in this volume. The human T-cell lymphotropic (T-cell leukaemia/lymphoma) viruses (HTLV) are enveloped viruses with a diameter of approximately 80–100 nm (Figure 1). The HTLV virions contain two covalently bound genomic RNA strands, which are complexed with the viral enzymes reverse transcriptase (RT; with associated RNase H activity), integrase and protease and the capsid proteins. The outer part of the virions consists of a membrane-associated matrix protein and a lipid layer intersected by the envelope proteins (Gelderblom, 1991).

Figure 1. An electron micrograph of HTLV-I virus

![HTLV-I virions](image)

Courtesy of Dr Bernard Kramarsky, Advanced Biotechnologies, Inc., Columbia, MD, USA

1.1.2 Taxonomy and phylogeny

Traditionally, retroviruses (family Retroviridae) have been classified according to a combination of criteria including disease association, morphology and cytopathic effects in vitro. On this basis three subfamilies were defined. The oncoviruses (Greek, *onkos* = mass, swelling) consist of four morphological subtypes which are associated with tumours in naturally or experimentally infected animals, and non-oncogenic related viruses. The second group, the lentiviruses (Latin, *lentus* = slow), cause a variety of diseases including immunodeficiency and wasting syndromes, usually after a long period...
of clinical latency. The third subfamily, the spumaviruses (Latin, spuma = foam), so called because of the characteristic ‘foamy’ appearance induced in infected cells in vitro, have not been conclusively linked to any disease. More recently, the International Committee on the Taxonomy of Viruses has divided the Retroviridae family into seven genera on the basis of genetic structure. The lentiviruses and spumaviruses each constitute a genus; the oncoviruses have been subdivided into five genera (Coffin, 1996). The HTLVs form one of these genera, along with the related bovine and simian viruses (see Section 1.1.4), and in turn can be divided into type I (HTLV-I) and type II (HTLV-II) according to their genetic composition and serotype. The genotypes of HTLV types I and II are related to each other; within these types, genetic variability is greater in the type I group. By the use of the polymerase chain reaction (PCR) and sequencing, strain variation within types has been characterized in viruses from humans residing in different geographical areas.

The term HTLV-III was assigned to a virus which was later defined as HIV-1 (see the monograph in this volume).

Three subtypes of HTLV-I (known as clades (Myers et al., 1993), which are defined as groups of viral strains with common nucleotides at any given position in the DNA sequence analysed) can be recognized using several analytical methods and by studying different viral genes (Figure 2) (Koralnik et al., 1994).

The cosmopolitan clade (HTLV-I\textsubscript{com}), found in many populations across the world (also known as HTLV-IA), represents a very homogeneous group of viruses. In the New World, HTLV-I\textsubscript{com} was probably introduced by the slave trade (Gallo et al., 1983; Gessain et al., 1992a; Koralnik et al., 1994) (see Figure 2).

The second clade (HTLV-I\textsubscript{Zaire}), also known as HTLV-IB, was identified in central African populations in the Zairian basin (Figure 2) (Gessain et al., 1992a).

The third clade (HTLV-I\textsubscript{Mel}) was identified in inhabitants of Papua-New Guinea and the Solomon Islands and later in Australian Aboriginals (Bastian et al., 1993; Gessain et al., 1993). Phylogenetic analysis has shown that this clade (also known as HTLV-IC) and HTLV-I\textsubscript{com} probably evolved independently from a common ancestor (Figure 2). Analysis of sequence variations among these viral strains suggests that the HTLV-I\textsubscript{Mel} clade diverged earliest, before the split between the HTLV-I\textsubscript{Zaire} and HTLV-I\textsubscript{com} groups.

Two other subgroups have been proposed within the HTLV-I\textsubscript{com} clade, but rigorous phylogenetic analysis does not appear to support this notion.

Phylogenetically, HTLV-II separates into three clades: Iia, Iib and Iic. HTLV-Iia and Iib can be further divided into several subgroups (Dube et al., 1993; Neel et al., 1994; Eiraku et al., 1995; Gessain et al., 1995a; Switzer et al., 1995; Biggar et al., 1996; Eiraku et al., 1996). Approximately 70% of HTLV-II from intravenous drug users has been found to be HTLV-Iia. Amerindian tribes from Central and North America have the distinct type Iib, whereas remote Amazonian tribes harbour mainly subtype Iia (Biggar et al., 1996; Eiraku et al., 1996). These findings suggest that ancestral Amerindians who migrated to the New World brought at least two and possibly three genetic subtypes of HTLV-II (Neel et al., 1994; Biggar et al., 1996; Eiraku et al., 1996).
1.1.3 Host range

HTLV-I and HTLV-II have been isolated from humans (Poiesz et al., 1980; Kalyanaraman et al., 1982). Under experimental conditions, both HTLV-I and HTLV-II infect rabbits (Miyoshi et al., 1985; Cockerell et al., 1991) and HTLV-I can also infect rats (Yoshiki et al., 1987; Ibrahim et al., 1994). Among non-human primates, HTLV-I isolates have been shown to infect rhesus macaques (Macaca mulatta) (Lerche et al., 1987), cynomolgus monkeys (M. fascicularis) and squirrel monkeys (Saimiri sciureus) (Yamamoto et al., 1984; Nakamura et al., 1986). (See also Section 3).
1.1.4 **Related non-human primate viruses**

Viruses related to HTLV have been isolated from non-human primates. These are known as simian T-cell lymphotropic viruses (STLVs). DNA analysis of STLV-I strains of African and Asian origin has led to several conclusions. STLV-I from a single species can be sorted into genetically distinct clades. The distribution of STLV-I phylogenetic clades from *Cercopithecus aethiops* (African green monkey), *Pan troglodytes* (the common chimpanzee) and *Papio* (baboon) (respectively S3 and S6; S2 and S5; S4 and S7 in Figure 2) indicates that these retroviruses did not evolve within each species and suggests interspecies transfer within the primate genera, including man.

The human HTLV-I 

The human HTLV-I and the common chimpanzee clade S5 are closely related (Figure 2), suggesting that the human clade may have resulted from cross-species transmission of chimpanzee STLV-I to humans. Two additional examples of interspecies transmission which are suggested by the phylogenetic analysis of STLV-I from African primates with different geographical origins (Koralnik et al., 1994) are shown in Figure 2. In the equatorial region of Africa, the STLV-I clades S2, S3 and S5 and HTLV-I 

The S1 clade, from Asia, contains heterogeneous members and is closely related to HTLV-I 

These results indicate the evolution of three clades in the human species and suggest that at least three independent introductions of HTLV-I into humans occurred during the evolution of these retroviruses. A simple interpretation of the global dissemination of these retroviruses might be the following. Ancestors of HTLV-I and STLV-I entered primates in Asia and were transmitted to several species. Primates infected with STLVs migrated to Africa, where the viruses were transmitted to local primate genera (*Cercopithecus, Papio, Pan* and humans). Meanwhile, HTLV-I emerged by a separate primate-to-human transfer in Melanesia. More recent human migratory patterns, including the slave trade, led to the dissemination of the cosmopolitan HTLV-I clade worldwide. This hypothesis implies the existence of STLV for over 30 000 000 years, at least since the end of the Oligocene epoch and the beginning of the Miocene era, when the continents were linked, favouring contacts between primate species (Martin, 1990).

The recent description of STLVs in two species of African primates, the pygmy chimpanzees (*Pan paniscus*) and baboons from Ethiopia, adds further complexity to our picture of the evolution of the STLVs and HTLVs. Two closely related viruses (Giri et al., 1994; Liu et al., 1994) isolated from pygmy chimpanzees that live exclusively in central Africa (Kano, 1984; de Waal, 1995) are nearer to HTLV-II than to HTLV-I (Giri et al., 1994; Liu et al., 1994). This finding, in addition to the discovery of sporadic cases of HTLV-II infection in human pygmies (Goubau et al., 1992; Gessain et al., 1995a), raises questions concerning the origin and evolution of HTLV-II, previously thought to be a New World virus. Another STLV, designated primate T-lymphotropic virus-L (PTLV-L) (Goubau et al., 1994), appears to be phylogenetically equidistant between HTLV-I and HTLV-II.
The observations of interspecies transmission of these phylogenetically distinct viruses among non-human primates (Saksena et al., 1994) (see Section 3.2) and of indeterminate serological profiles of HTLVs (see Section 1.2) found in some human populations raise the question of the existence of other HTLV-related viruses in addition to HTLV-I and HTLV-II in humans (see also Figure 3).

Figure 3. Phylogenetic analysis of HTLV-I/STLV-I and HTLV-II/STLV-II

The DNA sequence of a 522 bp envelope fragment from various STLV and HTLV strains was used in a neighbour-joining analysis to define their phylogenetic relationship.

Adapted from Koralnik et al. (1994)
HTLV-I infects CD4\(^+\) T-cells and occasionally CD8\(^+\) T-cells \textit{in vitro} (Markham et al., 1983; Popovic et al., 1983) and, less efficiently, other cells including macrophages, B-cells and glial cells (Longo et al., 1984; Hoffman et al., 1992; Koralnik et al., 1992a). \textit{In vivo}, HTLV-I is mainly, if not exclusively, associated with CD4\(^+\) T-cells (Richardson et al., 1990). HTLV-II infects mainly CD8\(^+\) T-cells \textit{in vitro} and almost exclusively CD8\(^+\) T-cells \textit{in vivo} (Rosenblatt et al., 1988a; Hall et al., 1994).

### 1.1.6 Genomic structure and properties of gene products

The HTLV-I genome (Seiki et al., 1983) of approximately 9 kb encodes structural proteins (Gag and Env), enzymes (RT, integrase and protease) and regulatory proteins (Tax and Rex). The two long terminal repeats (LTR) located at the 5' and 3' ends of the viral genome contain the viral promoter and other regulatory elements. HTLV-I increases its complexity by alternative splicing of viral messenger ribonucleic acid (mRNA) in the region at the 3' end of the genome known as pX (Seiki et al., 1985; Aldovini et al., 1986; Nagashima et al., 1986; Furukawa et al., 1991; Orita et al., 1991; Berneman et al., 1992a; Koralnik et al., 1992b; Orita et al., 1993), which contains at least four open-reading frames (Gitlin et al., 1993), and possibly by the use of an internal promoter (Nosaka et al., 1993). The regulatory proteins Tax and Rex are derived from this region. Rex, a post-transcriptional regulator of viral expression (Kiyokawa et al., 1985; Hidaka et al., 1988; Inoue et al., 1991), and Tax, the viral transactivator of transcription (Sodroski et al., 1984; Cann et al., 1985; Felber et al., 1985), are both encoded by double-spliced polycistronic mRNAs in open reading frames III and IV.

Tax, the 42 kDa viral transactivator, is a nuclear phosphoprotein which exerts its effect on the Tax-responsive elements (TRE-1 and TRE-2) located in the U3 region of the viral LTR (Sodroski et al., 1984; Felber et al., 1985). Tax does not bind directly to TRE-1 or TRE-2, but activates other transcriptional factors which do so. Members of the cyclic AMP (c-AMP)-responsive element-binding proteins and activating transcription factor (CREB/ATF) family (leucine zipper protein) have been shown to interact with TRE-1 (a 21-bp repeated element) (Jeang et al., 1988a; Willems et al., 1992a; Suzuki et al., 1993; Adam et al., 1994), whereas TRE-2 contains binding sites for other transcriptional factors such as Sp1, TIF-1, Ets1 and Myb (Bosselut et al., 1990; Gitlin et al., 1991; Bosselut et al., 1992; Franchini, 1995) (reviewed in Gitlin et al., 1993; Yoshida, 1994). In addition to this complex transactivation of the viral LTR U3 region, Tax also positively transactivates cellular genes. Tax-mediated transactivation pathways and the resulting effects on cellular gene expression are discussed in detail in Section 4.3.

Rex (Kiyokawa et al., 1985; Nagashima et al., 1986; Hidaka et al., 1988; Inoue et al., 1991), generated by the same double-spliced mRNA that encodes Tax, is a 27 kDa nucleolar phosphoprotein which regulates the balance of single- and double-spliced versus unspliced viral mRNAs necessary for viral replication. Rex stimulates the expression of both the single-spliced mRNA for the envelope gene and the unspliced viral genomic RNA for the Gag/Pol proteins. However, it inhibits the splicing and transport of double-spliced mRNAs which encode for Rex itself, Tax and the other
alternatively spliced mRNAs in the pX region. The effect of Rex on mRNA level is exerted in trans on the cis-acting Rex response element (Rex RE), a highly stable RNA stem-loop structure in the U3/R region of the 3' LTR (Seiki et al., 1985; Yoshida & Seiki, 1987; Hanly et al., 1989). Since the Rex RE stem structure is present in all viral mRNAs, the differential regulation of spliced versus unspliced mRNA by Rex also relies on other cis elements in the viral genome (Black et al., 1994a). Rex also stabilizes the mRNA for the interleukin (IL)-2Rα chain by acting in trans on the coding sequence of the IL-2Rα chain gene (Kanamori et al., 1990), as well as indirectly potentiating IL-2 gene expression in concert with Tax (McGuire et al., 1993).

1.1.7 Other genes encoded by open reading frames I, II and III in the HTLV-I pX region

The double-spliced mRNA that encodes Tax and Rex also encodes another protein, p21\textsuperscript{\text{p31}}\textsuperscript{\text{p31}}, a cytoplasmic protein of unknown function (Furukawa et al., 1991), that has been identified in several HTLV-I-infected cell lines. Transcripts for p21\textsuperscript{\text{p31}}\textsuperscript{\text{p31}} have been found to be highly expressed also in uncultured adult T-cell leukaemia/lymphoma (ATLL) samples (Berneman et al., 1992b). Three other proteins are encoded by alternative splicing of the pX region and transcripts for these mRNAs have been demonstrated in infected cells in vitro and in ex-vivo samples from healthy individuals as well as from patients with ATLL and tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) (Berneman et al., 1992b; Ciminale et al., 1992; Koralnik et al., 1992b; Orita et al., 1993). Double- and single-spliced mRNAs from open reading frame I encode a single protein of 12 kDa (p12\textsuperscript{\text{p31}}) in transfected cells (Koralnik et al., 1993).

The p13\textsuperscript{\text{p31}}\textsuperscript{\text{p31}} and p30\textsuperscript{\text{p31}} proteins, encoded by open reading frame II in the pX region, are expressed in the nucleus and nucleoli, respectively, of transfected cells (Koralnik et al., 1993). Neither p13\textsuperscript{\text{p31}}\textsuperscript{\text{p31}} nor p30\textsuperscript{\text{p31}} influences the activity of the regulatory genes tax and rex (Roithmann et al., 1994).

Four additional proteins are expressed in HTLV-II from open-reading frames I, II, III and V (Ciminale et al., 1995). Schematic representations of the most recent genomic maps of HTLV-I and HTLV-II are presented in Figure 4.

Spliced genes from the pX region of the bovine leukaemia virus (BLV) have also been described. This distant relative of HTLV causes B-cell leukaemia in cattle (Alexandersen et al., 1993; Kettmann et al., 1994), of which the clinical stages mirror those of HTLV-I-induced ATLL in humans (see Section 3.3). BLV encodes genes functionally equivalent to Tax and Rex and other proteins from the pX region. In a leukaemogenic BLV molecular clone, deletion of the R3 and G4 open reading frames (which are topologically equivalent to HTLV-I open reading frames I and II) results in an attenuated viral phenotype in vivo (Willems et al., 1994) (see also Section 3.3.2). Whether there is a biological relationship between these proteins encoded by the BLV and HTLV-I pX region is uncertain. In the case of HTLV-II, deletion of the region encoding these proteins but sparing the tax and rex genes does not alter its ability to immortalize T-cells in vitro (Green et al., 1995).
1.2 Methods of detection

The confirmed presence of HTLV-I or HTLV-II antibodies is considered to represent current infection, because, as with other human retroviruses, once acquired, infection is lifelong. This has been confirmed by virological and molecular studies.

1.2.1 Serological detection of specific antibodies

Two successive steps are generally necessary to demonstrate the presence of specific antibodies against HTLV-I or HTLV-II in serum, plasma, cerebrospinal fluid or other body fluids (Verdier et al., 1990; Lal & Heinene, 1996). The first is a screening assay, while the second is a confirmatory test which can also discriminate between antibodies directed specifically against HTLV-I or HTLV-II. The screening assays include enzyme-linked immunosorbent assay (ELISA), particle agglutination assay and immunofluorescence. All three methods can be used quantitatively (Gessain et al., 1988).

Commercial ELISA tests use, either alone or in combination, disrupted purified virions or specific peptides and recombinant proteins of HTLV-I or HTLV-II (Chen et al., 1990; Lillehoj et al., 1990; Lal et al., 1991; Washitani et al., 1991; Bonis et al., 1993; Rudolph et al., 1993, 1994; Lal, 1996). The specificity and sensitivity of these assays have been defined (Kline et al., 1991; Wiktor et al., 1991; Cossen et al., 1992; Karopolous et al., 1993; Jang et al. 1995). The use of additional specific peptides or recombinant proteins in earlier assays has increased their specificity.

The particle agglutination test uses gelatin particles sensitized with HTLV-I antigens (Ikeda et al., 1984; Fujino et al., 1991).
The indirect immunofluorescence test uses HTLV-I- and HTLV-II-producing cell lines as antigens (Aoki et al., 1985; Gallo et al., 1991).

Confirmatory assays use western blot, radioimmuno-precipitation and immunofluorescence. All commercial western blots contain disrupted purified HTLV-I virions (Gallo et al., 1994a). Generally, HTLV-I and HTLV-II induce antibodies directed against Gag proteins (p19 and p24) and their p53 precursor and Env glycoproteins (gp21 and gp46). Due to significant differences between HTLV-I and HTLV-II in the sequences of p19 and p24, HTLV-I-infected serum generally exhibits a stronger reactivity against p19 than against p24, while the opposite is true for HTLV-II (Wiktor et al., 1990). Some of these western blot assays have been supplemented by the addition of native gp46 specific to HTLV-I or recombinant gp21, recognized by both anti-HTLV-I and anti-HTLV-II antibodies (Lal et al., 1992a,b; Kleinman et al., 1994; Hadlock et al., 1995). HTLV-I and HTLV-II antibodies can be discriminated by the addition of synthetic specific peptides from the gp46 of HTLV-I (MTA1) and HTLV-II (K55) (Lipka et al., 1990; Hadlock et al., 1992; Lipka et al., 1992; Roberts et al., 1993).

A WHO working committee (WHO, 1990) proposed that confirmation of HTLV-I seropositivity must be based upon reactivity both to at least one gag-encoded protein (p19, p24) and to one or two env-encoded glycoproteins (gp21, gp46). However, more stringent criteria for HTLV-I and HTLV-II serodiagnosis have been proposed (HTLV European Research Network, 1996).

Indirect immunofluorescence has been used as a confirmatory assay to discriminate between HTLV-I and HTLV-II infection (Gallo et al., 1991).

Radioimmuno-precipitation is more sensitive than western blot, but is rarely used as a confirmatory assay because it is time-consuming, expensive and uses radioactive material. It has been useful in the detection of gp21 and gp46 seroreactivities in some unusual sero-indeterminate western blot patterns (Aboulafia et al., 1993; Gallo et al., 1994b).

Most of the immunoglobulins detected are IgG (Lal et al., 1993), but IgA and IgM can also be detected at certain periods of infection (Robert-Guroff et al., 1981; Manns et al., 1991, 1994).

Several algorithms have been used for the detection and confirmation of HTLV-I- or HTLV-II-positive serum specimens at blood banks in Japan (Aoki et al., 1985), the United States (Busch et al., 1994) and Europe (Tosswill et al., 1992; Taylor, 1996). However, some of the assays used for screening are less sensitive for HTLV-II than for HTLV-I and several studies have shown that HTLV-II may go undetected in blood donors (Hjelle et al., 1993; Weiss, 1994; Zehender et al., 1996).

### 1.2.2 Detection and characterization of viral nucleic acids

HTLV-I and HTLV-II are mainly cell-associated viruses. PCR allows the direct detection of proviral DNA sequences of HTLV-I or HTLV-II in cellular DNA (Ehrlich et al., 1990), which is usually obtained from peripheral blood mononuclear cells (PBMCs) found not only in blood but also in semen, breast milk and other body fluids (Iwahara et al., 1990). Primer pairs specific for HTLV-I and/or HTLV-II have been
developed from the pol and tax regions (Ehrlich et al., 1990). The genetic variability of both HTLV-I and HTLV-II is sufficiently low to permit the detection of the great majority of the existing viral strains.

Single-round PCR with 30/35 cycles can detect specific HTLV-I or HTLV-II proviral sequences in the DNA of PBMCs of persons with ATLL or TSP/HAM and in most healthy carriers. However, nested PCR is required for the detection of HTLV-I proviral sequences in a few individuals with a low viral level. The viral DNA can be sequenced either directly after PCR (Komurian et al., 1991) or after cloning in one of several possible vectors (Gessain et al., 1993). A simpler method to determine HTLV-I or HTLV-II viral subtype involves restriction fragment length polymorphism (RFLP) analysis of either LTR or the env gene (Ureta-Vidal et al., 1994).

The clonal integration of provirus(es) in ATLL cells can be demonstrated by Southern blot analysis (Yamaguchi et al., 1984) and/or inverse PCR (Takemoto et al., 1994).

In-vivo expression of HTLV-I or HTLV-II viral antigens is very low. Detection of viral RNA can generally be achieved only by very sensitive methods such as RT/PCR or in-situ hybridization (Gessain et al., 1991). In-situ PCR has recently been applied to HTLV-I infection (Levin et al., 1996).

Quantification of the proviral copy number in the DNA of PBMCs can be achieved by several techniques (Tachibana et al., 1992; Matsumura et al., 1993; Cimarelli et al., 1995; Miyata et al., 1995; Morand-Joubert et al., 1995) (see Section 4.3.1).

1.2.3 Isolation of HTLV-I and HTLV-II

Culture, in the presence of IL-2, of PBMCs from HTLV-I- or HTLV-II-infected individuals can lead, usually after several months, to the establishment of long-term T-cell lines which are either CD4+ or CD8+ cells expressing markers of activation (CD25, HLA-DR) (Gessain et al., 1990a; Dezzutti et al., 1993). These clonal T-cell lines, which can also be established by co-culture of PBMCs with phytohaemagglutinin-stimulated cord blood, produce viral particles, visible by electron microscopy, and viral antigens, as demonstrated by specific immunofluorescence using either polyclonal or monoclonal antibodies directed against p19 (Robert-Guroff et al., 1981), p24 (Gessain et al., 1990b) or gp46 (Edouard et al., 1994). These cell lines release viral Gag antigens into the culture supernatant, detectable by an antigen capture assay. The use of the BJAB cell line is very useful to isolate HTLV-II from cultured PBMCs of a patient co-infected with HTLV-II and HIV (Hall et al., 1992).

1.2.4 Sero-indeterminate HTLV-I western blots

There are difficulties in interpreting some western blots of HTLV-I or HTLV-II in serum specimens, particularly those from tropical areas (Weber et al., 1989; Verdier et al., 1990; Gessain et al., 1995a). A high percentage of western blots of specimens from equatorial Africa and Melanesia exhibit indeterminate patterns, with reactivities to ‘gag-encoded proteins’ p19, and/or p24 and/or p53 and/or proteins of uncertain origin (p26, p28, p32 and p36), but without reactivity to Env glycoproteins gp21 and gp46 (Garin et al., 1994). As a consequence, a number of studies have overestimated the
HTLV-I seroprevalence in these regions (Biggar et al., 1985; Brabin et al., 1989; Garin et al., 1994). In an effort to standardize results, more stringent criteria for western blot positivity have been proposed by WHO (1990) and by the Centers for Disease Control and Prevention (CDC) (1992).

With commercial HTLV-I western blot kits that contain only low amounts of native glycoprotein (gp21, gp46), only persons with high HTLV-I titres, such as patients with TSP/HAM, exhibit a clear Env reactivity. Despite significant progress in specificity of western blot assays, some problems remain; for example, the low specificity of seroreactivity directed against the recombinant Env gp21 leads to false-positive interpretations. A modified version of this recombinant antigen with higher specificity is now available (Varma et al., 1995). The WHO and the CDC diagnostic guidelines need to be further validated for samples originating from tropical areas (Gessain et al., 1995b).

1.2.5 Seronegative HTLV-I-infected individuals

A few individuals have been described who are seronegative for both HTLV-I and HTLV-II, but in whom fragments of HTLV-I provirus in their PBMCs have been detected by PCR. In some West Indian HTLV-I-seronegative patients with a clinical TSP/HAM syndrome, some investigators have demonstrated the presence of HTLV-I-related sequences in their PBMC DNA. In most such cases, the detected sequences were small fragments of the tax and/or pol genes. Recently, an HTLV-I-seronegative TSP/HAM patient harbouring a defective HTLV-I virus in his PBMCs was reported (Daenke et al., 1994). However, most studies indicate that, in healthy individuals, this is very rare, even in HTLV-I endemic areas. Thus, several studies performed in Japan, in the Caribbean region and in the United States have failed to detect HTLV-I proviral sequences in the DNA of PBMCs from seronegative subjects, even children born to HTLV-I-seropositive parents. The possibility of a cryptic infection in which HTLV-I resides elsewhere than in the peripheral blood remains, however, a possibility.

The issue of detection of proviral HTLV sequences in seronegative patients with cutaneous T-cell lymphomas other than ATLL is discussed in Section 2.1.2.

1.3 Epidemiology of HTLV infection

1.3.1 HTLV-I transmission

Three modes of transmission have been demonstrated for HTLV-I.

(a) Mother-to-child transmission

Mother-to-child transmission represents a major mode of transmission of HTLV-I in endemic areas, mainly due to breast-feeding beyond six months (Hino, 1990a; Tajima et al., 1990a; Takahashi et al., 1991; Monplaisir et al., 1993; Wiktor et al., 1993; Hino et al., 1994), after which time the protective IgG maternal antibodies decline (Takahashi et al., 1991). Seroconversion (the development of detectable specific antibodies to the virus in the serum) in children occurs between 18 and 24 months of age (Takahashi et al., 1991). Depending on the population studied, 10–25% of breast-fed children from
HTLV-I-seropositive mothers become infected with the virus (Ando et al., 1987; Hino et al., 1987a,b; Hino, 1990a; Tajima et al., 1990a; Takahashi et al., 1991; Ando et al., 1993; Monplaisir et al., 1993; Hino et al., 1994). This transmission is linked to the presence of HTLV-I provirus in mononuclear cells in breast milk (Kinoshita et al., 1984, 1985a). Maternal factors associated with transmission, which correlate with high HTLV-I viral load, are: high HTLV-I antibody titres directed against the whole virus, presence of anti-Tax antibodies and in-vitro maternal HTLV-I antigen expression in short-term culture (Sugiyama et al., 1986; Hino et al., 1987a; Sawada et al., 1989; Kashiwagi et al., 1990; Wiktor et al., 1993). Other factors include the presence of antibodies directed against certain immunogenic epitopes of the gp46 envelope glycoprotein and maternal age > 30 years (Wiktor et al., 1993).

Strong evidence that breast-feeding plays the predominant role in mother-to-child transmission comes from Japanese studies in which advice to HTLV-I-seropositive mothers not to breast-feed their babies resulted in a significant decrease in mother-to-child transmission of the virus, albeit with unexplained regional variation (Ando et al., 1987; Hino et al., 1987a; Tsuji et al., 1990; Hino et al., 1994; Katamine et al., 1994). Thus in Nagasaki prefecture, the risk of the maternal transmission was reduced from 20–30% to 3% by bottle feeding (Hino et al., 1994; Takezaki et al., 1996), whereas in Okinawa prefecture about 13% of bottle-fed children (all under 10 years of age and none transfused) born to carrier mothers were infected by HTLV-I. Evidence against transplacental transmission comes from a study in which none of seven children with HTLV-I proviral DNA-positive cord blood cells seroconverted by 24–8 months. The observation that none of the cord blood samples of nine formula-fed children, who were later confirmed to be infected, was positive for HTLV-I suggests that intrauterine infection was not the cause of viral transmission (Katamine et al., 1994).

There are no data on the role of vaginal delivery in HTLV-I transmission.

(b) Sexual transmission

HTLV-I is sexually transmissible and this transmission is more efficient from men to women than the reverse (Tajima et al., 1982; Kajiyama et al., 1986; Stuver et al., 1993; Take et al., 1993; Figueroa et al., 1995; Takezaki et al., 1995). The risk for transmission, over 10 years, from seropositive husbands to wives has been calculated at 60%, whereas that for transmission from wives to husbands was only 0.4% (Kajiyama et al., 1986). Another study reported that over 50% of the wives of HTLV-I seropositive husbands were infected within one to four years after marriage (Take et al., 1993). Female prostitutes of Fukuoka (Japan) had a significantly higher seroprevalence of HTLV-I antibodies than various control populations (Nakashima et al., 1995). In prostitutes in Peru, HTLV-I seropositivity was linked to duration of prostitution, lack of consistent condom use and past infection with Chlamydia trachomatis (Wignall et al., 1992; Gotuzzo et al., 1994). In a group of 409 Zairian prostitutes from Kinshasa, the annual incidence of HTLV-I was 0.7% (Delaporte et al., 1995). Risk factors for HTLV-I infection in Jamaican women attending sexually transmitted disease clinics included multiple sexual partnership, a current diagnosis of syphilis and the presence of other venereal diseases (Murphy et al., 1989a). Further strong evidence for sexually transmitted infection comes from a
prospective study of 600 subjects over the age of 40 years tested during 1976–93; eight seroconverted, of whom five had an HTLV-I-seropositive spouse and two seroconverted after blood transfusions (Takezaki et al., 1995). In Europe, HTLV-I-infected blood donors, who are usually female, are almost always from an endemic area or have had sexual intercourse with a person from an HTLV-I endemic area (The HTLV Europe Research Network, 1996; Taylor, 1996). Seroconversion in the female partner of a transplant recipient infected by blood transfusion has also been documented (Gout et al., 1990).

(c) Transmission by blood

Infection by blood transfusion appears to be the most efficient mode of HTLV-I transmission, with a 15–60% risk of infection among recipients of a contaminated cellular blood product (Okochi et al., 1984; Inaba et al., 1989; Manns et al., 1991; Sandler et al., 1991; Manns et al., 1992; Donegan et al., 1994). Fresh frozen plasma, which is acellular, is not infectious. Platelets are more likely than red blood cells to transmit HTLV-I infection when transfused, probably because they are more heavily contaminated by T lymphocytes (Okochi et al., 1984; Lairmore et al., 1989; Manns et al., 1991; Sandler et al., 1991; Manns et al., 1992). Infectivity decreases with increasing duration of storage at 4 °C, a temperature at which lymphocyte survival is reduced. In a study in Jamaica, immunosuppressive therapy at the time of transfusion was found to increase the risk of HTLV-I seroconversion (Manns et al., 1992).

In highly endemic areas such as southern Japan and the West Indies, with a 0.5–5% HTLV-I seroprevalence among blood donors (Gessain et al., 1984; Minamoto et al., 1988), multi-transfused patients (Barbara, 1994) including renal transplant recipients (Linhares et al., 1994) have high HTLV-I seroprevalence. Screening of blood donations has been implemented in Japan (Maeda et al., 1984; Okochi et al., 1984), French Guiana and the Caribbean islands of Martinique and Guadeloupe (Massari et al., 1994; Pillonel et al., 1994), the United States (Williams et al., 1988; Lee et al., 1991; Sandler et al., 1991), Canada, France (Couroucé et al., 1993; Massari et al., 1994; Pillonel et al., 1994) and Denmark (Bohn Christiansen et al., 1995) and Netherlands during the last decade. The issues in relation to testing blood donors in other European countries have been discussed (Salker et al., 1990; Brennan et al., 1993; Soriano et al., 1993; Taylor, 1996). In areas of low endemicity (0.002–0.02% among blood donors) such as metropolitan France and the United States, HTLV-I seropositivity among donors is associated mainly with birth in highly endemic regions (such as the West Indies) or with having sexual partners from endemic areas. In various African and South American countries, where HTLV-I seroprevalence in blood donors ranges from 0.2% to 1%, it has been suggested that compulsory HTLV-I screening of donors should be considered (Gutfraind et al., 1994; Ferreira et al., 1995).

Transmission of both HTLV-I and HTLV-II between intravenous drug users has been documented, with a higher rate for HTLV-II than for HTLV-I (Hall et al., 1994; Schwebke et al., 1994; Hall et al., 1996).
1.3.2 Animal models of HTLV-I transmission

Experiments have demonstrated that HTLV-I can be transmitted to and infect several species of monkeys (Yamamoto et al., 1984; Nakamura et al., 1986), rabbits (Miyoshi et al., 1985; Cockerell et al., 1991) and rats (Ibrahim et al., 1994) by either intravenous or intraperitoneal inoculation of autologous or heterologous HTLV-I-transformed cell lines. HTLV-I infection of rabbits or marmosets has been effected by intravenous or oral inoculation of HTLV-I-transformed and virus-producing cells (Kinoshita et al., 1985a; Yamanouchi et al., 1985; Uemura et al., 1986; Iwahara et al., 1990). Inoculation of rabbits with cell-free concentrated HTLV-I virions led to only a transient seroconversion, without detectable virus remaining after a few months (Miyoshi, 1994). Experimental transmission of HTLV-I by blood transfusion and from mother to offspring has also been observed in rabbits (Uemura et al., 1986; Iwahara et al., 1990); as little as 0.01 mL of infected blood (corresponding to $1.7 \times 10^4$ lymphocytes) was capable of transmitting the virus. Hori et al. (1995) have demonstrated intrauterine transmission of HTLV-I in rats, albeit at a low rate.

1.3.3 Geographical distribution of HTLV-I

HTLV-I is not a ubiquitous virus but is spread throughout the world with small clusters of hyperendemicity located within endemic areas (Levine et al., 1988; Mueller, 1991; Blattner & Gallo, 1994). Information on seroprevalence has been based on surveys of highly variable size and quality and may not be reliable. Only in Japan have population-based studies been conducted. In endemic areas, the HTLV-I antibody prevalence in the adult population varies from 0.2% to 15% (see Figure 5). Based on strict diagnostic criteria using confirmatory assays (western blot; WHO, 1990; Gessain & Mathieux, 1995) and/or specific immunofluorescence (Gallo et al., 1991), low HTLV-I seroprevalence refers to seropositivity in adults ranging from 0.2% to 2%, while higher rates in adults define highly endemic areas. The latter include the south-western islands of Japan, the Caribbean, South America, intertropical Africa, parts of the Middle East (Iran) and Melanesia.

In the Far East, the Japanese islands of Okinawa, Kyushu and Shikoku represent highly endemic areas, with an estimated one million HTLV-I carriers (Tajima et al., 1982; Hino et al., 1984; Ishida et al., 1985; Hinuma, 1986; Tajima et al., 1986, 1987; Kosaka et al., 1989; Tajima & Hinuma, 1992; Morofuji-Hirata et al., 1993; Tajima et al., 1994; Brodine et al., 1995). Most other parts of Japan have lower seroprevalence. The rest of the Far East region has a low level of HTLV-I endemicity, with sporadic cases reported in Taiwan (Wang et al., 1988; Chen et al., 1994), in some areas of India (Babu et al., 1993; Singhal et al., 1993), China (Pan et al., 1991), Korea (Lee et al., 1986), Nepal (Ishida et al., 1992) and the Philippines (Ishida et al., 1988). Few data are available relating to Siberia (Gessain et al., 1996a) or Mongolia (Batsuuri et al., 1993), but sporadic cases of HTLV-I infection in individuals living in the central part of Sakhalin island have been reported (Gurtsevitch et al., 1995; Gessain et al., 1996a). In spite of some reported cases of HTLV-I infection, circumpolar populations cannot be considered as endemically infected (Robert-Guroff et al., 1985; Davidson et al., 1990).
Figure 5. Estimated percentage of HTLV-I carriers among blood donors ≥ 40 years) in Japanese prefectures in 1983

From Maeda et al. (1984)

Africa is often considered to be the largest reservoir for HTLV-I infection. It has been estimated that 5–10 million individuals may be infected (Hunsmann et al., 1984; Saxinger et al., 1984; Verdier et al., 1994), in most tropical countries including Benin, Burkina-Fasso, Equatorial Guinea, Ghana, Guinea, Guinea Bissau, Ivory Coast, Mali, Nigeria, Senegal and Tchad in west Africa (Biggar et al., 1984; Hunsmann et al., 1984; Saxinger et al., 1984; de Thé et al., 1985; de Thé & Gessain, 1986; Delaporte et al., 1989a; Ouattara et al., 1989; Verdier et al., 1989; Dumas et al., 1991; Biggar et al., 1993; Dada et al., 1993; Del Mistro et al., 1994; Verdier et al., 1994; Jeannel et al., 1995) and Cameroon, Central African Republic, the Congo, Gabon and Zaire in central Africa (Hunsmann et al., 1984; Saxinger et al., 1984; Delaporte et al., 1989b; Goubau et al., 1990; Delaporte et al., 1991; Schrijvers et al., 1991; Goubau et al., 1993a; Garin et al., 1994; Maucere et al., 1994; Tuppin et al., 1996). While most of these countries exhibit low HTLV-I seroprevalence overall, areas of high prevalence have been detected in southern Gabon (Delaporte et al., 1989, 1991; Schrijvers et al., 1991) and northern Zaire (Goubau et al., 1990, 1993a; Garin et al., 1994). In north Africa (El-Farrash et al., 1988; Farouqi et al., 1992), east and South Africa (Hunsmann et al., 1984; Saxinger et al., 1984; Bhigjee et al., 1990, 1993; Verdier et al., 1994) and Indian Ocean islands (Mahieux et al., 1994), the level of endemicity seems very low, despite occasional clusters, such as in the Seychelles (Román et al., 1987).

In the Americas, highly endemic areas include the Caribbean islands of Haiti, Jamaica, Martinique and Trinidad (Schaffar-Deshayes et al., 1984; Clark et al., 1985a; Miller et al., 1986; Gibbs et al., 1987; Riedel et al., 1989; Blattner et al., 1990; Fréry et al., 1991; Maloney et al., 1991; Murphy et al., 1991; Ramirez et al., 1991; Allain et al., 1992; Manns et al., 1992; Miller et al., 1994) and limited areas of South America such as Tumaco in Colombia (Trujillo et al., 1992) and the Noir-Marron territory in
French Guiana (Gessain et al., 1984; Gérard et al., 1995; Tuppin et al., 1995). Low-level HTLV-I endemicity has been reported in large regions of Latin America (Ohtsu et al., 1987; Maloney et al., 1989; Cevallos et al., 1990; Pombo de Oliveira et al., 1990; Zamora et al., 1990; Guerena-Burgueno et al., 1992; Duenas-Barajas et al., 1993). In the United States and Canada, prevalence is low except in Afro-Americans and in recent immigrants from endemic areas (Weinberg et al., 1988; Williams et al., 1988; Khabbaz et al., 1990; Chadburn et al., 1991; Eble et al., 1993; Dekaban et al., 1994; Harrington et al., 1995).

There is no known HTLV-I endemic area in Europe; early reports from southern Italy (Manzari et al., 1985) are disputed (de Stasio et al., 1989; Chironna et al., 1994) and most cases of HTLV-I infection have been in immigrants from the West Indies, Africa or the Middle East, or in persons who had sexual relationships with such immigrants (Cruickshank et al., 1989; Wyld et al., 1990; Taylor, 1996). However, sporadic cases of HTLV-I infection without evidence of a link with an endemic area have been reported in Greece (Dalekos et al., 1995), Romania (Paun et al., 1994), Georgia (Senjuta et al., 1991), Sicily (Boeii et al., 1995; Mansueto et al., 1995) and the United Kingdom (Wyld et al., 1990).

While in European countries the great majority of HTLV-seropositive blood donors are infected with HTLV-I (Taylor, 1996), in the United States (Lee et al., 1991), 60–70% are infected with HTLV-II.

In the Middle East, the Mashhad region in northern Iran appears to be an important reservoir of HTLV-I infection (Achiron et al., 1993; Nerurkar et al., 1995), with seropositive emigrants from this region now living in Israel, the United States (Meytes et al., 1990) and northern Italy (Achiron et al., 1993). Furthermore, sporadic cases of HTLV-I infection have been reported in Iraq (Denic et al., 1990) and Kuwait (Voevodin et al., 1995).

In the Pacific region, isolated clusters of HTLV-I have been described, especially in two tribes of Papua New Guinea (Garruto et al., 1990; Yanagihara et al., 1990; Lal et al., 1992c; Nerurkar et al., 1992; Yanagihara, 1994) and in the Australian Aboriginal population (May et al., 1988; Bastian et al., 1993; Bolton et al., 1994). Furthermore, HTLV-I is endemic in the Solomon Islands (Garruto et al., 1990; Yanagihara et al., 1991), but seems very rare in most other Pacific islands (Garruto et al., 1990).

The origin of this puzzling geographical clustering is not well understood, but is probably linked to a founder effect in certain communities, with persistence due to a putatively high mother-to-child transmission of the virus under favourable environmental and cultural conditions (Tajima et al., 1990a; Mueller, 1991; Kaplan & Khabbaz, 1993; Blattner & Gallo, 1994; Tajima et al., 1994). Such clustering linked to the background of the population has been studied in French Guiana (Tuppin et al., 1995): among 1873 pregnant women (the HTLV-I serological status could be established for 1716 of them), the HTLV-I seroprevalence rate differed significantly between ethnic groups: 5.7% for Noir-Marron (70/1302), 6.3% for Haitian (3/50) and 0% for Creole (0/126), Amerindians (0/166) and Hmong (0/64). Thus, the Noir-Marron, descendants of fugitive slaves of African origin, with limited contact with other groups, represent a major reservoir for
HTLV-I infection (Gessain et al., 1984; Gérard et al., 1995; Tuppin et al., 1995). In Trinidad, among a sample of persons selected from a government register, 3.2% of 1025 persons of African descent were HTLV-I-seropositive compared with 0.2% among 487 persons of Asian descent, while the prevalence of HTLV-I infection was 11.4% among persons of African ancestry in a coastal village of Tobago (Blattner et al., 1990).

1.3.4 **HTLV-I prevalence and demographic features of HTLV-I infection**

It has been estimated that worldwide between 15 and 20 million individuals are infected with HTLV-I, with 2–10% developing an HTLV-I-associated disease during their lifetime (de Thé & Bomford, 1993; Blattner & Gallo, 1994) (described in Sections 1.4 and 2.1). In highly endemic areas, and despite widely different socioeconomic and cultural environments, the HTLV-I seroprevalence is low and stable among children but increases gradually with age, most markedly in women over 50 years of age, but also in men (Tajima & Hinuma, 1984; Tajima et al., 1987; Maloney et al., 1991; Mueller, 1991; Murphy et al., 1991; Blattner & Gallo, 1994). Several explanations for this significant age-dependent increase in HTLV-I-seroprevalence in women have been proposed. First, it could be the result of an accumulation of sexual exposure with increasing age. However, for most sexually transmitted infections, transmission occurs mainly during the period when sexual activity is at its peak (Mueller, 1991). Second, the apparent age-dependence may be confounded by a cohort effect (Blattner et al., 1986; Chavance et al., 1989; Ueda et al., 1989; Chavance & Fréry, 1993; Takezaki et al., 1995), suggested in some but not all cross-sectional surveys in Japan. Finally, these infections in older persons might be due to reactivation of silent infection which becomes apparent on account of immuno-dysregulation that occurs with aging. However, several studies using PCR methods have failed to detect proviral DNA sequences in the PBMCs of HTLV-I-seronegative healthy individuals (Nakashima et al., 1990). Thus, there is at present no consistent explanation for the excess prevalence among older people (Figure 6).

In Kumamoto (Japan), the annual age- and sex-specific HTLV-I carrier prevalence in blood donors below 50 years of age declined between 1986 to 1990 in both sexes, and it has been suggested that the HTLV-I carrier state of individuals below the age of 50 years will become negligible in southern Japan within the first half of the next century (Oguma, 1990; Oguma et al., 1992, 1995).

1.3.5 **Epidemiology of tropical spastic paraparesis/HTLV-I-associated myelopathy**

The etiological link between HTLV-I and tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) is based on: (1) observations of very high prevalence (up to 90%) of HTLV-I infection in patients with TSP, (2) the occurrence of TSP/HAM following transfusion with HTLV-I-contaminated blood and (3) the decreased incidence of TSP/HAM in transfusion recipients after the introduction of blood donor screening for HTLV-I in Japan (Gessain et al., 1985; Osame et al., 1986a,b; Gout et al., 1990; Kaplan et al., 1990).
The association between HTLV-I and tropical spastic paraparesis (TSP) in the French West Indies (Martinique) was described in 1985 (Gessain et al., 1985) and was soon confirmed in Jamaica (Rodgers-Johnson et al., 1988) and in Colombia (Rodgers-Johnson et al., 1985), and subsequently in Japan, where the same clinical entity was named HTLV-I-associated myelopathy (HAM) by Osame et al. (1986a). TSP/HAM is more frequent in women (sex ratio ranging from 1:1.5 in Japan to 3.5 in Martinique) and is common in most HTLV-I endemic areas, but is very rare in children (Osame et al., 1986a; Román et al., 1987; Shibasaki et al., 1989; Kaplan et al., 1990; Kayembe et al., 1990; Janssen et al., 1991; Ramiandrisoa et al., 1991; Gessain & Gout, 1992; Jeannel et al., 1993), where it can be one of the major neurological diseases. Reliable estimates of TSP/HAM incidence and prevalence are available only for Japan, some Caribbean areas and rare clusters in Africa and South America (Gessain & Gout, 1992). Thus, the prevalence of TSP/HAM ranges from 8.6/100,000 inhabitants in Kyushu (Japan) (Shibasaki et al., 1989; Kaplan et al., 1990; Osame et al., 1990) to 128/100,000 in Mahé (Seychelles) (Gessain & Gout, 1992). Estimates of the annual incidence range from 0.04/100,000 in Kyushu (Shibasaki et al., 1989; Kaplan et al., 1990) to 3/100,000 in Lisala (Zaire) (Kayembe et al., 1990). The female predominance seems to be less marked in South America (Araújo et al., 1993), where TSP/HAM affects all racial groups (Araújo et al., 1993; Rodgers-Johnson, 1994; Domingues et al., 1995).

The prevalence of TSP/HAM varies greatly across geographical areas despite similar levels of HTLV-I seropositivity (Román et al., 1987; Kaplan et al., 1990; Kayembe et al., 1990; Trujillo et al., 1992; Jeannel et al., 1993). Thus, southern Japan and Martinique have similar seroprevalence of HTLV-I, but in Martinique, the prevalence of TSP/HAM among HTLV-I carriers (around 250 cases among 6000–10,000 HTLV-I carriers in a total population of 333,000) is estimated at 1.5–3%, while in Japan,
the prevalence of TSP/HAM in HTLV-I-infected persons is estimated to be only 0.08% (Kaplan et al., 1990).

Within a particular geographical area, the prevalence of TSP/HAM can vary according to ethnic group. Thus in Inongo, Zaire (Jeannel et al., 1993), among the five major ethnic groups, the Bolia exhibit the highest prevalence of HTLV-I (6.5%) without any detected TSP/HAM cases, while six TSP/HAM cases were found among the Ntomba, whose HTLV-I prevalence rate was only 2.2%. Such findings suggest that, besides HTLV-I infection, environmental and/or genetic cofactors play a part in the development of TSP/HAM.

People infected with HTLV-I through blood transfusion have a higher risk for developing TSP/HAM than people infected by other means (Gout et al., 1990; Osame et al., 1990). In Japan and Martinique, up to 20% of TSP/HAM patients had a blood transfusion in the five years preceding onset of the disease. In the first two years of screening of the blood supply in Japan for HTLV-I, started at the end of 1986, a 16% decrease in patients with TSP/HAM was reported (Osame et al., 1990). Direct evidence for a causal relationship between HTLV-I and TSP/HAM was obtained when a seronegative cardiac graft recipient seroconverted 14 weeks after an HTLV-I-positive blood transfusion and, four weeks later, exhibited a severe disorder of the pyramidal tract identical to that seen in TSP/HAM. HTLV-I was isolated from mononuclear cell cultures from his peripheral blood and from his cerebrospinal fluid (Gout et al., 1990).

In contrast, development of ATLL after HTLV-I infection by blood transfusion seems extremely rare, if it exists (Williams et al., 1991).

1.3.6 Natural history of HTLV-I primary infection

Among the several dozen documented cases of HTLV-I seroconversion, no acute seroconversion illness has been reported. Following infection with HTLV-I by blood transfusion, viral IgG-specific antibodies are detectable within one to four months in most cases. In the first two months after infection, antibody to Gag protein predominates, with anti-p24 generally appearing before anti-p19. Antibody to recombinant gp21 is frequently the earliest Env reactivity detected, with anti-gp46 appearing later. Anti-Tax antibodies appear much later (Manns et al., 1991, 1994). In the first three months, IgM are the most frequent isotypes, although IgG and IgA can also be detected. HTLV-I-specific antibody responses persist in all Ig isotypes during the next four to six months and remain for many years (Manns et al., 1994).

1.3.7 Molecular epidemiology of HTLV-I

Based on sequence and/or RFLP analysis of more than 250 HTLV-I isolates originating from the main viral endemic areas, three major clades have emerged (Gessain et al., 1996b). Between the three clades (HTLV-I\textsubscript{com} or HTLV-I\textsubscript{A}, HTLV-I\textsubscript{zaire} or HTLV-I\textsubscript{B} and HTLV-I\textsubscript{med} or HTLV-I\textsubscript{C} (see Section 1.1.2), depending on the gene, the nucleotide changes range from 0.5 to 10%. DNA sequence analyses indicate that, within the three clades, there exist molecular subgroups clearly defined by several specific mutations, but these are not always consistent with phylogenetic analyses. For example, there is evi-
idence for two ancestral HTLV-I lineages in Japan (Mahieux et al., 1995): the classical cosmopolitan genotype, that represents around 25% of the Japanese HTLV-I and is found mainly in the southern islands, and another related subgroup called the ‘Japanese’ group, that differs at the nucleotide level by around 1.6% in the LTR and is evenly distributed in the Japanese archipelago (Ureta-Vidal et al., 1994). Similarly, within the central African clade (HTLV-I\textsubscript{zai}), there are molecular subgroups defined by specific substitutions in either the Env or the LTR sequences.

1.3.8 HTLV-II epidemiology

In 1982, HTLV-II was isolated from a cell line derived from the splenic cells of a patient with a lymphoproliferative disease originally considered to be a ‘T variant of hairy-cell leukaemia’ (Kalyanaraman et al., 1982).

While the modes of transmission of HTLV-II appear to be basically the same as those of HTLV-I, the global distribution of HTLV-II is very different. HTLV-II is highly endemic among some scattered Amerindian tribes including the Navajo and Pueblo in New Mexico, the Seminole in Florida, the Guaymi in Panama, the Cayapo (Kayapo) and Kraho in Brazil, the Wayu and Guahido in Colombia and the Tobas and Matacos in northern Argentina (Heneine et al., 1991; Gabbai et al., 1993; Black et al., 1994b; Bouzas et al., 1994) (reviewed in Hall et al., 1994, 1996). In these populations, HTLV-II seroprevalence varies greatly but can reach 20% of the general adult population and up to 50% in women aged over 50 years, as in Cayapo groups living in Brazil (Black et al., 1994b). HTLV-II also appears to be endemic in some pygmy tribes from Zaire and Cameroon (Goubau et al., 1993b) (reviewed in Gessain et al., 1995a; Gessain & de Thé, 1996), in contradiction to the earlier idea that HTLV-II was exclusively a ‘New World virus’ brought to the Americas by migrations of infected Mongoloid populations, who were the ancestors of the present-day Amerindians.

In aboriginal groups, mother-to-child transmission of HTLV-II through breast-feeding and sexual transmission appears to be important (Black et al., 1994b). In the developed countries, HTLV-II is found almost exclusively in intravenous drug users and their sexual partners (Tedder et al., 1984; Lee et al., 1989; Zella et al., 1990; Parry et al., 1991; Khabbaz et al., 1992; Al et al., 1993; Coste et al., 1993; Blomberg et al., 1994; Vallejo & Garcia-Saiz, 1994; Henrard et al., 1995); transmission occurs mainly through sharing contaminated needles (among intravenous drug users) (Lee et al., 1989; Khabbaz et al., 1992) and blood transfusion (Lee et al., 1991).

1.4 Clinical description of non-neoplastic disorders

1.4.1 HTLV-I infection

(a) Tropical spastic paraparesis/HTLV-I-associated myelopathy

TSP/HAM is a progressive form of chronic spastic myelopathy associated with demyelination of the spinal cord motor neurons (Gessain et al., 1985; Osame et al., 1986a; Dalgleish et al., 1988; Salazar-Grueso et al., 1990; Cruickshank et al., 1992; Araújo et al., 1993; Domingues et al., 1995; Harrington et al., 1995). It usually has an
insidious onset but rare cases of more rapid onset have been described, particularly following blood transfusion. The main clinical manifestations are weakness and stiffness of the lower limbs, urinary bladder disturbances, paraesthesias, lumbar pain and impotence. Difficulty in walking develops several months after presentation (Cruickshank et al., 1992; Rodgers-Johnson, 1994; St Clair Morgan, 1994). Cerebellar signs, cranial nerve palsies and convulsions are rare. Neurological examination reveals spasticity and/or hyperreflexia and muscle weakness in the lower extremities; half of the patients have mild sensory abnormalities. Objective clinical criteria for the diagnosis of TSP/HAM have been published by a WHO working group (WHO, 1989). [The diagnosis of TSP/HAM requires differentiation from multiple sclerosis, spinal cord compression, spinal canal stenosis and cervical spondylisis.]

The main immuno-virological features of TSP/HAM are: the presence of high titres of anti-HTLV-I antibodies in serum and cerebrospinal fluid (Dalgleish et al., 1988); pleocytosis in the cerebrospinal fluid with intrathecal IgG synthesis with oligoclonal bands that react with HTLV-I (Gessain et al., 1988); a high proviral load in the PBMCs (Yoshida et al., 1989; Gessain et al., 1990b); ex-vivo spontaneous lymphoid proliferation (Itoyama et al., 1988); circulating activated T-cell subpopulations (Minato et al., 1989; Shibayama et al., 1992) and presence of cytotoxic T-cells which recognize epitopes of the products of the tax gene (Jacobson et al., 1990). All of these features except those in the cerebrospinal fluid have been described in asymptomatic carriers.

A small number of circulating abnormal ‘flower-like’ lymphocytes similar to those of ATLL are present in about half of the patients (Dalgleish et al., 1988; St Clair Morgan, 1994).

Magnetic resonance imaging may reveal abnormalities in the white matter of the brain and electrophysiology often demonstrates latency delays of visual, brainstem auditory and somatosensory evoked potentials with normal peripheral nerve conduction (St Clair Morgan, 1994).

Histological data are derived mainly from post-mortem examinations. The pathological changes affect the grey and white matter of the spinal cord, particularly the lateral columns; the brain is grossly normal and the leptomeninges are thickened (St Clair Morgan, 1994). The main features are marked demyelination and axonal destruction with an inflammatory mononuclear-cell infiltrate; astrocytic gliosis and meningeal thickening are common. By immunohistology, perivascular infiltrating mononuclear cells are T cells, mainly CD4+ at early stages and CD8+ in the later stages. Macrophages may also be found. HTLV-I has been detected in the nervous tissue by PCR with primers against pol, env and pX genes and by in-situ hybridization (Kira et al., 1991, 1992a; Kira, 1994; Kuroda et al., 1994; Sueyoshi et al., 1994; Umehara et al., 1994), but there is no direct evidence that HTLV-I infects neurons in vivo (St Clair Morgan, 1994) and it is uncertain which cell type (T lymphocytes, microglia or neural cells) is infected.

The clinical course of TSP/HAM is progressive. Oral corticosteroids may produce a transient beneficial effect, particularly when given in the early phases of the disease. Other drugs including azathioprine, danazol, intrathecal hydrocortisone and α-interferon...
can provide temporary relief. In addition, symptomatic treatment with diazepam or dantrolene can be used to relieve spasticity (St Clair Morgan, 1994).

The pathogenesis of TSP/HAM is uncertain, but viral load, specific molecular viral strain, specific and non-specific immune response and human leukocyte antigen (HLA) variability have been considered as potential factors in disease development (Gessain & Gout, 1992; Bangham, 1993; Bangham et al., 1996). Extensive sequence studies mainly of the LTR region and the \textit{env} gene have failed to define any specific nucleotide changes linked to disease (Mahieux et al., 1995). Although Tax-specific cytotoxic T-cells were first described in the PBMCs of TSP/HAM patients (Jacobson et al., 1990), their prevalence and frequency have been reported to be the same in TSP/HAM cases and asymptomatic carriers (Parker et al., 1994; Daenke et al., 1996). Central nervous system (CNS) inflammation is characterized by perivascular infiltration of lymphocytes (mainly CD8$^+$), but HTLV-I is rarely detected in the CNS. It has therefore been suggested that the CNS damage may be a non-specific consequence of T-cells activated by HTLV-I leaving the circulation and causing bystander damage (Bangham et al., 1996).

(b) \textit{Uveitis}

Uveitis is an inflammatory condition of the uveal tract. The majority of cases of uveitis are idiopathic, but some are caused by bacterial or viral infections and some are associated with autoimmune diseases, such as in Behçet’s syndrome. Idiopathic uveitis in Japan is more frequent in HTLV-I endemic areas, such as southern Kyushu, and the seroprevalence of HTLV-I in these patients is significantly higher (up to 38\%) than in patients with uveitis of other known etiologies (Mochizuki et al., 1992, 1994). Because the seroprevalence of HTLV-I is much higher in young patients, it has been suggested that early exposure to the virus, such as at birth, is important in the development of uveitis (Mochizuki et al., 1994).

HTLV-I-associated uveitis affects younger adults, usually under 50 years of age, and can be uni- or bilateral. It has a subacute onset, presenting with blurred vision but with little or no decrease in visual acuity. The main physical sign of HTLV-I uveitis is vitreous opacity (Mochizuki et al., 1994). The course is progressive in the absence of treatment with topical or systemic corticosteroids. Recurrence of uveitis is common but remission may last for years (Ohba et al., 1994). Familial occurrence of HTLV-I uveitis has been described (Araki et al., 1993), as well as its association with TSP/HAM and hyperthyroidism (Nakao et al., 1994; Ohba et al., 1994).

An association between HTLV-I-associated uveitis and Graves’ disease has been reported, evoking speculation that thyroid hormones may modify the host response to the virus and/or activate viral replication (Mochizuki et al., 1994).

Inflammatory cellular infiltrates with HTLV-I-infected cells are present in the ocular tissues. Infiltrating lymphocytes in the vitreous and aqueous humour contain integrated proviral \textit{tax} gene (Mochizuki et al., 1994; Sagawa et al., 1995) and express mRNA for HTLV-I proteins (Sagawa et al., 1995). These lymphocytes display an activated T-cell phenotype (CD3$^+$, CD4$^+$, CD25$^+$) and release a variety of cytokines (such as ILs and tumour necrosis factor (TNF) $\alpha$), which may be responsible for the inflammation
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(Sagawa et al., 1995). Sequencing of the LTR region of HTLV-I has shown that uveitis is not associated with a specific viral strain (Ono et al., 1994). Although the etiopathogenesis is unknown, the evidence available supports an autoimmune mechanism mediated by HTLV-I-activated T cells.

(c) Other inflammatory disorders

Patients with TSP/HAM additionally have inflammation in tissues other than the CNS, that is characterized by infiltration with activated T-lymphocytes and antibodies in the relevant body fluids. HTLV-I has been detected by molecular methods in these tissues, usually in lymphocytes, but epidemiological data linking HTLV-I with these conditions are weaker than for TSP/HAM. In particular, there is ascertainment bias, with most conditions initially described in patients with TSP/HAM. These inflammatory disorders have also been reported in HTLV-I infected persons without TSP/HAM.

(i) Infective dermatitis

Infective dermatitis, an exudative dermatitis affecting the scalp, ears, axillae and groin, characterized by the presence of non-pathogenic bacteria, has been almost exclusively reported in HTLV-I-seropositive Jamaican children (LaGrenade et al., 1990), with an average age at onset of two years. These children require long-term antibiotic therapy. There is mild lymphocytosis in peripheral blood with an increase in CD4+ cells and often polyclonal hypergammaglobulinaemia. Retrospective analysis has suggested that children with infective dermatitis may be at increased risk of later developing TSP/HAM or ATLL (Bunker et al., 1990; Pagliuca et al., 1990; Hanchard et al., 1991; LaGrenade, 1994).

(ii) Polymyositis

Polymyositis is an inflammatory myopathy characterized by proximal muscle weakness and wasting, raised serum levels of muscle enzymes (serum lactate dehydrogenase, creatine kinase and aminotransferase) and distinct histological changes. The cause is largely unknown, although some cases are linked to autoimmune disease or infections with viruses such as Coxsackie B. In HTLV-I-endemic areas, the prevalence of HTLV-I antibodies in patients with polymyositis has been found to be substantially higher than in corresponding control groups (85% against 8%: St Clair Morgan et al., 1989; 28% against 11.6%: Higuchi et al., 1992) (reviewed in Dalakas, 1993). HTLV-I-positive polymyositis affects women more frequently than men, appears to be more common in Caribbean than in Japanese patients and seems to be associated with TSP/HAM (St Clair Morgan et al., 1989; Smadja et al., 1993; Sherman et al., 1995). Both IgM and IgG HTLV-I antibodies are detected in most cases. Electromyography shows changes consistent with an inflammatory myopathy, such as short duration of polyphasic motor unit potentials (St Clair Morgan et al., 1989; Sherman et al., 1995).

Histological features are those of a myositis (Sherman et al., 1995), with atrophy, necrosis, oedema, fibrosis and interstitial cellular infiltrates composed of macrophages and lymphocytes (mainly CD8+ with some CD4+). HTLV-I sequences have not been found in the muscle cells (Higuchi et al., 1992; Sherman et al., 1995).
(iii) Alveolitis

In the original description, asymptomatic alveolitis was found at bronchoalveolar lavage (BAL) in patients with TSP/HAM (Sugimoto et al., 1987). Asymptomatic alveolitis may occur in HTLV-I carriers and in patients with HTLV-I-associated uveitis (Maruyama et al., 1988; Sugimoto et al., 1993). However, a few patients have a persistent cough and/or a variable degree of dyspnoea. The chest X-ray is usually normal, but localized or patchy reticular shadows, pleural thickening and/or lung fibrosis have been described. Antibodies to HTLV-I are detected in both serum and BAL fluid. BAL fluid may contain lymphocytes predominantly of the CD4+ phenotype (Maruyama et al., 1988) or have a normal distribution of CD4+ and CD8+ cells (Sugimoto et al., 1993) and display an immune response to HTLV-I (Maruyama et al., 1988). An increased number of activated T cells (CD3+, CD4+ or CD8+) expressing HLA-DR determinants and CD25 have been found in the blood and BAL fluid from these patients (Mukae et al., 1994). Patients with alveolitis associated with TSP/HAM have increased soluble IL-2 receptors in the BAL fluid (Sugimoto et al., 1989). Patients with alveolitis associated with uveitis have increased viral load in both blood and BAL fluid (Sugimoto et al., 1993).

(iv) Arthritis (HTLV-I-associated arthropathy)

HTLV-I-associated arthropathy is a chronic inflammatory oligoarthritis of large joints, which preferentially affects middle-aged or elderly female HTLV-I carriers (Nishioka et al., 1989; Ijichi et al., 1990; Nishioka et al., 1993) and is often associated with TSP/HAM (Kitajima et al., 1989). Antibodies to HTLV-I are detected in both the serum and synovial fluid. Most patients have IgG antibodies, but up to two thirds also have IgM antibodies, suggesting active replication of the virus in the synovial fluid. Rheumatoid factor and features of autoimmune disease are usually absent and X-rays of the affected joints show marginal erosions and narrowing of the joint spaces (Kitajima et al., 1989). Arthroscopy reveals synovial proliferation, while mild changes in the cartilage and subchondral bone are seen histologically, with mononuclear infiltrates composed of lymphocytes with multilobulated nuclei (Nishioka et al., 1989, 1993). Immunostaining demonstrates HLA-DR expression by the synovial cells and by lymphocytes that are mainly CD4+ and CD8+ T-cells expressing retroviral proteins (Nishioka et al., 1993). By PCR, HTLV-I proviral sequences have been detected in both lymphocytes and synovial cells purified by T-cell depletion (Kitajima et al., 1991). Cultured synovial cells express mRNA for HTLV-I tax/rex as well as HTLV-I core and envelope proteins, as detected by immunostaining (Kitajima et al., 1991; Nishioka et al., 1993). In vitro studies have also demonstrated that synovial cells are susceptible to infection by HTLV-I, proliferate vigorously and produce large amounts of granulocyte-macrophage colony-stimulating factor (Sakai et al., 1993).

The importance of HTLV-I tax in the pathogenesis of this condition is supported by studies in which transgenic mice with the HTLV-I pX gene develop a similar polyarthritis (Iwakura et al., 1991; Yamamoto et al., 1993). Whether this is mediated by HTLV-I-infected lymphocytes secreting cytokines which stimulate the proliferation of synovial cells or by a direct stimulation of synovial cells by HTLV-I is unknown.
(v) Thyroiditis

An association between HTLV-I infection and Hashimoto's thyroiditis (inflammation of the thyroid gland with autoantibodies) has been reported from Japan (Kawai et al., 1991, 1992; Smadja et al., 1993; Mizokami et al., 1995). The seroprevalence of HTLV-I in these patients was significantly higher than that in the corresponding general population (6.3% versus 2.2%) (Kawai et al., 1992). This condition is often found in patients with TSP/HAM (Kawai et al., 1991, 1992) and uveitis (Mizokami et al., 1995).

(vi) Sjögren's syndrome

Sjögren’s syndrome, a keratoconjunctivitis, with dryness of the eyes and mouth and hypertrophy and lymphocytic infiltration of the salivary glands, has been observed in HTLV-I carriers and in patients with HTLV-I-associated diseases (Merle et al., 1994; Eguchi et al., 1992; Plumelle et al., 1993). As with other inflammatory diseases associated with HTLV-I, there is an increase in circulating activated cells (CD3+, CD25+, HLA-DR) that display spontaneous proliferation (Eguchi et al., 1992). In some HTLV-I-seronegative patients with Sjögren's syndrome, HTLV-I tax but not pol, gag and env sequences have been detected in labial salivary glands (Mariette et al., 1993, 1994; Sumida et al., 1994).

Transgenic mice with the HTLV-I tax gene have been shown to develop a condition similar to Sjögren's syndrome. Lymphocytic infiltration of the salivary glands with the presence of tax in the epithelial cells has been demonstrated in these mice (Green et al., 1989a).

(d) Immune suppression

T-cell subsets and CD4/CD8 ratios do not appear to be affected by HTLV-I infection (Matutes et al., 1986; Welles et al., 1994).

Evidence of mild immune suppression due to HTLV-I infection has been seen in studies of healthy carriers who had decreased delayed hypersensitivity to the purified protein derivative of tuberculin (Tachibana et al., 1988) and marked suppression of T-cell control of B cells infected with Epstein-Barr virus (EBV) (Katsuki et al., 1987). Indirect evidence of impaired cellular immunity has come from studies showing that HTLV-I carriers have a reduced ability to clear infection with Strongyloides stercoralis (Nakada et al., 1987). S. stercoralis infection is associated with ATLL and, when present, is often severe (Nakada et al., 1987; Dixon et al., 1989; Phels et al., 1991; Patey et al., 1992; Plumelle et al., 1993).

1.4.2 HTLV-II infection

HTLV-II has occasionally been associated with a myeloneuropathy resembling TSP/HAM with ataxia (Hjelle et al., 1992; Murphy et al., 1993; Harrington et al., 1993; Murphy et al., 1996). Few studies have attempted to investigate the association between HTLV-II and diseases in populations in which the infection is endemic.
1.4.3  *HTLV/HIV co-infection*

Co-infection with HIV and HTLV-I or HTLV-II is common among HIV-1-infected intravenous drug users and patients attending clinics for sexually transmitted diseases in areas where both viruses are endemic (Harper *et al.*, 1986; Wiley *et al.*, 1989; Manzari *et al.*, 1990; Khabaz *et al.*, 1992; Beilke *et al.*, 1994; Harrington *et al.*, 1995). Although the clinical consequences of the co-infection are largely unknown, it has been suggested that HTLV-I but not HTLV-II may accelerate the course of HIV-1 infection. Patients with HTLV-I and HIV-1 co-infection develop specific HIV-1-related disease manifestation at higher CD4+ T-cell count than patients with HIV-1 infection only (Beilke *et al.*, 1994; Harrington *et al.*, 1995; Schechter *et al.*, 1994).

There have been some case reports of co-infected persons developing either associated haematological disease or inflammatory disease (Harper *et al.*, 1986).

1.5  Control and prevention

Prevention of HTLV-I and HTLV-II infection must be directed at the main modes of HTLV-I transmission: perinatal, especially postnatal though breast-feeding; parenteral, through blood transfusion or exposure to contaminated needles; and sexual, essentially male to female (Hino, 1990b; Sato & Okochi, 1990; Bentrem *et al.*, 1994).

Prevention of HTLV-I infection in neonates appears to be particularly important because of the association of ATLL with childhood infection. Maternal antibodies may protect infants during short-term (less than six months) exposure but, as this wanes, susceptibility to infection appears to increase (Takahashi *et al.*, 1991). An intervention programme to screen and counsel HTLV-I-seropositive mothers against breast-feeding began in Japan in the late 1980s and has been shown to prevent 90% of maternal infection of infants (Hino, 1990b). More recently, these recommendations have been changed to permit short-duration breast-feeding. Such a policy can be adopted only where safe and sustainable alternatives to breast-feeding are available.

Transmission of HTLV-I in cellular blood products is highly efficient, with a seroconversion rate of 63% (Bentrem *et al.*, 1994), and TSP/HAM and other inflammatory HTLV-I-linked diseases develop within a relatively short time following blood transfusion (Osame *et al.*, 1986b). Transfusion-related transmission can be prevented by systematic screening of blood donors for HTLV-I and HTLV-II antibodies, as is practised in several countries (see Section 1.3.1). Infection of a health worker with HTLV-I by puncture with a contaminated needle has been known to occur (Bentrem *et al.*, 1994), emphasizing the need for universal precautions in dealing with biological materials.

Use of condoms during sexual intercourse should be considered by couples when only one partner is infected with HTLV-I or HTLV-II.

Passive immunization has been shown to be effective in rabbits: hyperimmune IgG prepared from seropositive healthy persons given 24 h before transfusion with infected blood appeared to protect the recipient rabbit from infection (Takehara *et al.*, 1989; Kataoka *et al.*, 1990).
Although such policies may help to control the spread of HTLV-I and HTLV-II, the ideal intervention would be immunization with a preventive vaccine. Preclinical studies in animal models have suggested the feasibility of an HTLV-I vaccine. Various live recombinant pox virus vectors carrying the HTLV-I envelope protein have conferred protection against a cell-associated HTLV-I challenge in non-human primates (Shida et al., 1987) and rabbits (Franchini et al., 1995). Certain live recombinant envelope proteins alone have also conferred protection in non-human primates (Nakamura et al., 1987). However, no trials of HTLV-I vaccines in humans have yet been undertaken.