REVIEW ARTICLE

Viral Aetiology of Adult T-Cell Leukaemia

By NAOKI YAMAMOTO1 AND YORIO HINUMA2*

1Department of Virology and Parasitology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755 and 2Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606, Japan

INTRODUCTION

RNA tumour viruses (retroviruses) have been implicated as the causal agents of many naturally occurring neoplasms such as sarcomas, leukaemias, malignant lymphomas and carcinomas in various animal species (Aaronson & Stephenson, 1976; Hardy et al., 1980). Evidence for an involvement of retroviruses in human malignancies, however, has been obtained only very recently. In 1980 and 1981, human T-cell leukaemia virus (HTLV) and adult T-cell leukaemia (ATL) virus (ATLV) were isolated independently in the U.S.A. from an aggressive type of T-cell malignancy and in Japan from a patient with ATL, respectively (Poiesz et al., 1980, 1981; Hinuma et al., 1981; Yoshida et al., 1982). Both viruses exhibited close similarities to the hitherto known animal retroviruses but were different structurally and immunologically (Rho et al., 1981; Reitz et al., 1981; Kalyanaraman et al., 1981). These two viruses quickly attracted the attention of a number of virologists and haematologists and the aetiological role of these viruses in mature T-cell malignancy was investigated extensively. Sufficient results accumulated to suggest that the viruses were indistinguishable (Watanabe et al., 1984; Yoshida et al., 1984).

Though ATL represents only a minor fraction of all human malignancies it is extremely important that a retrovirus has been found which may cause a human malignancy. The discovery of HTLV/ATLV and the determination of its causal role in ATL provides an invaluable model for investigation of human malignancies by various means, e.g. the therapy and immunoprevention of leukaemia, and the possible involvement of viruses in other human malignancies.

ATL

From statistical investigations carried out in the early 1970s it was apparent that the frequency of T-cell lymphoma/leukaemia is very high in Japan, especially in Kyushu Island (Akazaki & Wakasa, 1974). In 1977 Takatsuki and his colleagues first described ATL as a new disease entity among the T-cell malignancies (Takatsuki et al., 1977; Uchiyama et al., 1977). The disease is summarized as an acute or subacute T-cell leukaemia occurring in adulthood (40 or more years old) with (i) no sexual difference in occurrence, (ii) a high frequency of cutaneous infiltration, (iii) hepatosplenomegaly and swelling of lymph nodes, (iv) no apparent bone marrow infiltration, (v) hypercalcaemia, (vi) appearance of characteristic cells of leukaemia with convoluted nuclei, (vii) no mediastinal tumours. The most characteristic feature of the disease, however, is that ATL patients cluster in Kyushu Island, south-western Shikoku and the southern Kii Peninsula. Up to now about 1000 cases of ATL have been reported and it is estimated that about 200 new cases are discovered each year.

Leukaemic cells in fresh peripheral blood samples from ATL patients possess the surface markers of mature T-cells, that is they are OKT-3+, OKT-4+, OKT-5−, OKT-6−, OKT-8− and Ia+ (Hattori et al., 1981). ATL cells exert an inhibitory effect on immunoglobulin production by pokeweed mitogen-stimulated B-cells although they carry the surface markers of helper T-cells (Hattori et al., 1981).
Fig. 1. Detection of ATLA in MT-1 and MT-2 cells by indirect immunofluorescence (IF). Acetone-fixed cells were treated with test serum from a patient with ATL and fluorescein-labelled anti-human IgG. Note the 3 to 5% of fluorescent cells in MT-1 (left) but 100% in MT-2 cells (right). Because of this, MT-1 cells are suitable for IF studies.

The geopathological distribution of ATL, which occurs at high frequency in south-western Japan which has rather mild temperatures in winter and is hot and humid in summer, is reminiscent to some extent of that of Burkitt’s lymphoma (BL) in Africa (Burkitt, 1958). BL clusters in black children along the equatorial belt. Epstein–Barr virus (EBV) has been isolated from cell lines originating from these patients and an aetiological role of this virus in BL is likely (Epstein & Achong, 1979). Thus it was quite reasonable for Japanese researchers to consider the involvement of an infectious agent, possibly a virus, in the pathogenesis of ATL.

**ATL cell lines and the discovery of HTLV/ATLV**

In 1979 Miyoshi et al. cultured peripheral blood lymphocytes (PBL) from a 69 year-old male with ATL and obtained a permanent lymphoblastoid cell line, MT-1 (Miyoshi et al., 1979). During establishment of the cells in culture they used as a feeder layer leukocytes containing macrophages with a male karyotype, derived from normal cord blood. MT-1 cells possessed the C14q+ marker chromosome that is also seen in primary leukaemic cells, indicating that the cell line was indeed leukaemic in origin. This cell line was clearly positive for T-cell markers and terminal deoxynucleotidyl transferase but lacking surface IgG, complement receptors and EBV-determined nuclear antigens (EBNA). Hinuma et al. discovered in 1981 that a specific antigen–antibody reaction could be detected by immunofluorescence when sera from ATL patients were reacted with this cell line after acetone fixation (Hinuma et al., 1981). Since no specific fluorescence was observed with control sera from healthy donors or from patients with other malignancies, they named the antigen in MT-1 cells ATL-associated antigen (ATLA) (Fig. 1). The number of ATLA-positive cells increased significantly when MT-1 cells were treated with iododeoxyuridine (I UdR) which is known to be a viral inducer in various systems. Under these conditions electron microscopy revealed the presence of C-type particles in MT-1 cells (Fig. 2). Although these particles resembled in morphology other known retroviruses from various species of animals they characteristically exhibited almost no budding stages (Hinuma et al., 1981). Moreover, their sizes varied greatly, ranging from 50 to 150 nm in diameter.
Miyoshi continued similar attempts to establish cell lines from ATL patients and succeeded in obtaining a second cell line, MT-2 (Miyoshi et al., 1981). In contrast to the derivation of the MT-1 cell line, PBL from a female patient with ATL were co-cultivated with cord blood lymphocytes (CBL) with male chromosomes. The resultant T-cell line grew with a doubling time of 30 to 40 h without the use of T-cell growth factor (TCGF) (Ruscetti & Gallo, 1981). Strikingly, the established cells carried the male sex chromosome, apparently indicating their origin from the normal lymphocytes used as the feeder cells. This experiment was the first demonstration of transforming activity associated with the leukaemic cells of ATL patients. Moreover, the MT-2 cell line spontaneously produced large amounts of C-type particles that were indistinguishable from those seen in MT-1 cells. Thus, it was suggested that transformation of normal lymphocytes could be attributed to these C-type viruses. Yoshida et al. (1982) carried out molecular biological studies with this cell line and demonstrated that the virus was a retrovirus; it was designated ATLV.
On the other hand the HUT-102 cell line, from which HTLV was isolated in 1980, was established in the presence of TCGF from a biopsy specimen of lymph node from a 28 year-old black male patient (CR) with mycosis fungoides (Gazdar et al., 1980). HUT-102 cells did not produce detectable amounts of virus particles by electron microscopy initially. Another cell line designated CTCL-2 was established from PBL of a 69 year-old black female (MB) with Sezary syndrome (Poiesz et al., 1981). Both mycosis fungoides and Sezary syndrome occur sporadically all over the world and both are types of cutaneous T-cell leukaemia/lymphoma distinguishable from ATL. CTCL-2 and HUT-102 became independent of TCGF for growth after about the 10th passage. After treatment with IUdR these cell lines were found to produce a retrovirus, designated HTLV, detectable by both reverse transcriptase activity and electron microscopy. However, the cases of disease from which HTLV was first isolated are now recognized to be very similar, if not identical, to ATL (Posner et al., 1981; Gallo et al., 1983). Furthermore, both viruses have proved to be indistinguishable by immunological and genetic investigations (Watanabe et al., 1984).

**HTLV/ATLV**

**Biological activity**

Table 1 summarizes which cells and species HTLV/ATLV can infect and/or transform.

**Co-culture**

As described above, MT-2 was derived from the normal T lymphocytes of cord blood used as feeder cells in the growth of ATL leukaemic cells immortalized by ATLV infection (Miyoshi et al., 1981). Yamamoto et al. (1982b) studied this phenomenon more systematically using the MT-2 cell line itself. MT-2 cells were lethally irradiated and co-cultured with CBL and PBL. In co-cultures with irradiated MT-2 cells, transformation and continuous growth of ‘recipient’ cells from adults or newborns were observed after 2 to 4 weeks of incubation. All of the established cells were positive for ATLA and produced ATLV in the medium as revealed by electron microscopy. All the lines tested were negative for EBNA and immunoglobulins. However, they contained many cells with the T-cell surface markers Leu1, Leu3a and Leu4. Thus, most of the established cells were characterized as T-cells. These surface markers were very similar to those exhibited by leukaemic cells of ATL (Uchiyama et al., 1977; Hattori et al., 1981). Similar work was done in several other laboratories, in some cases using fresh PBL from ATL patients and from healthy carriers, and the results were found to be reproducible (Popovic et al., 1983; Markham et al., 1983). These newly established cell lines often constitutively liberated one or more of the various lymphokines such as γ-interferon, differentiation-inducing factor, phagocytosis-inducing factor, colony-stimulation factor, macrophage and leucocyte migration inhibitory factor, leucocyte migration-enhancing factor, TCGF, interleukin 3, platelet-derived growth factor and B-cell growth factor (Salahuddin et al., 1984; Hinuma et al., 1984).

**Cell-free viral infection**

A number of attempts were made to transform human leukocytes with cell-free virus, especially that from MT-2 cells. However, all these trials were unsuccessful (Yamamoto et al., 1982b). Chosa et al. (1982), on the other hand, reported that cell-free virus from MT-2 cell cultures infected and induced ATLA in the presence of phytohaemagglutinin in leukocytes from randomly selected healthy individuals without anti-ATLA antibody. Likewise, Ruscetti et al. (1983) reported that PBL from members of the family of patient CR who had no anti-HTLV antibody, were infectible by cell-free virus, whereas PBL from unrelated subjects were not. It was shown by means of membrane immunofluorescence that cell-free ATLV derived from MT-2 cells was able to adsorb to many haematopoietic cell lines from various species, such as man, mouse and marmoset, thus suggesting the presence of virus receptors in a wide range of animal tissues (Yamamoto et al., 1984a). As discussed more thoroughly in the following section, ATLV/HTLV can infect not only human lymphocytes but also non-human cells. Clapham et al. (1983) obtained a HTLV-positive cell line, HOS/PL, by the co-culture of the human osteogenic sarcoma line HOS with the HTLV-carrying C91/PL cell line. Cell-free virus derived from this
Table 1. Susceptibility of various cells to HTLV/ATLV infection

<table>
<thead>
<tr>
<th>Infection procedure*</th>
<th>Cells</th>
<th>Origin/cell type†</th>
<th>Species‡</th>
<th>Parameters of susceptibility§</th>
<th>Reference</th>
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<td>Syrian hamster</td>
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<td>T</td>
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<td>ATLA, Imm</td>
<td>19</td>
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</table>

* Coc, co-cultivation; Spon, spontaneously established cell line.
† N, non-T, non-B; OC, other cell type.
‡ AGM, African green monkey; JM, Japanese monkey; BM, bonnet monkey.
§ ATLA, ATL-associated membrane antigen; Syn, syncytium induction, Imm, immortalization.
||1, Chosa et al. (1982); 2, Yamamoto et al. (1984a); 3, Clapham et al. (1983); 4, Ho et al. (1984); 5, Miyoshi et al. (1981); 6, Yamamoto et al. (1982b); 7, Popovic et al. (1983); 8, Okada et al. (1984); 9, Nagy et al. (1983); 10, Hayami et al. (1984b); 11, Yoshikura et al. (1984); 12, Hoshino et al. (1983); 13, Hoxie et al. (1984); 14, Miyoshi et al. (1982a); 15, Miyoshi et al. (1983b); 16, Tateno et al. (1984); 17, Yamamoto et al. (1982a); 18, Yamamoto et al. (1984b); 19, M. Okada et al. (unpublished).¶ These cells have also been shown to be sensitive to plaque formation by VSV(HTLV-I) pseudotypes (Nagy et al., 1984).

Cell line could infect the HOS cell line in the presence of DEAE-dextran, with the first antigen-positive cells appearing at 21 days and their proportion increasing with time.

**Host range**

Although most of the cell lines established by co-culture of CBL or PBL with MT-2 cells were of T-cell lineage, some cell lines possessed so-called non-T, non-B cell surface markers, and lacked markers of either T- or B-cells (Yamamoto et al., 1982a). This was the first indication that
ATLV could infect non-T lymphoid cells. Yamamoto et al. (1982a) established seven continuous cell lines from PBL or lymph node biopsy specimens from ATL patients without the aid of TCGF or co-cultivation, and about half of them consistently expressed ATLA. They were all positive for EBNA and immunoglobulin, thus indicating their B-cell origin. Subcloning of these cell lines resulted in the development of EBNA-positive, ATLA-positive or EBNA-positive, ATLA-negative but not EBNA-negative, ATLA-positive clones (Yamamoto et al., 1982a; Koyanagi et al., 1984b). After enrichment of B-cells from PBL and co-cultivation with MT-2 cells, Okada et al. (1984) obtained a cell line from an EBV-seropositive donor, but not from an EBV-negative donor, which was again positive for both ATLA and EBNA. Similarly, B-cell lines dually infected with EBV and ATLV were obtained from the PBL of African green monkeys and Japanese monkeys (Yamamoto et al., 1984b; M. Okada et al., unpublished). Interestingly, Gallo et al. (1982) did not find HTLV homologous sequences in the B-cell lines derived from patient CR from whom the first HTLV was isolated. However, they reported later that some clones of a B-cell line established from an ATL patient did contain HTLV, though they were negative for EBV (Longo et al., 1984). That HTLV could be transmitted to animal cells was first shown by Miyoshi et al. (1982b) by the co-culture method. Japanese macaque lymphocytes and rabbit lymphocytes became positive for ATLA and eventually immortalized (Miyoshi et al., 1982b, 1983b). Similarly, rat lymphocytes were immortalized by this method (Tateno et al., 1984). Furthermore, the co-culture method revealed that non-lymphoid cells were the target for ATLV infection. Yoshikura et al. (1984) succeeded in transmitting HTLV to a human diploid cell strain (IMR90) using primary cultures of leukaemic cells from three out of four ATL patients. Interestingly, the cells of the patient giving the negative result contained only a defective viral genome. Hayami et al. (1984b) also reported successful transmission of ATLV to a number of human or non-human cells of various types. A variety of human and mammalian cell lines have been induced to form syncytia after co-cultivation with HTLV-producing cells (Nagy et al., 1983; Hoshino et al., 1983; Hayami et al., 1984b). Nagy et al. (1984) also showed that there is a broad range of cells susceptible to infection by pseudotypes of vesicular stomatitis virus (VSV) bearing the envelope glycoproteins of HTLV.

Molecular nature

Virus polypeptides

As has been described, the ATLA antigen was first discovered by indirect immunofluorescence. This antigen complex was subsequently analysed biochemically by immunoprecipitation with ATLA-specific antisera. It was shown that sera from ATL patients precipitated a polypeptide (p24) from purified ATLV obtained from [35S]methionine-labelled MT-2 cells (Yamamoto & Hinuma, 1982; Yoshida et al., 1982; Schneider et al., 1984a). This polypeptide is a major component of the virion. Determination of the amino acid sequence of the HTLV p24 revealed a statistically significant sequence homology with bovine leukaemia virus (BLV) (Oroszlan et al., 1982). Earlier, Kalyanaraman et al. had detected precipitation of p24 and p19 when iodine-labelled HTLV was reacted with sera from patients CR and NB, from whom HTLV had been derived, and the wife of CR (Kalyanaraman et al., 1981; Posner et al., 1981). Pulse–chase experiments with anti-p19 and anti-p24 antibodies raised in rabbits or with anti-p19 mouse monoclonal antibody strongly suggested that p53 is a precursor of p24 and p19 (Schneider et al., 1984b; Koyanagi et al., 1984a; Tanaka et al., 1983). Copeland et al. (1983) have reported the complete amino acid sequence of another gag gene product, p15. This protein has been shown to have an apparently evolutionary relationship with BLV p12, a basic DNA-binding protein.

The major glycopolypeptide immunoprecipitated with sera from ATL patients is gp68 in MT-2 cells (Yamamoto et al., 1983b). In addition, gp61 was detected as a minor glycoprotein (Koyanagi et al., 1984a). Most other ATLA-positive human cells, however, contain gp61 instead of gp68 (Koyanagi et al., 1984a; Sugamura et al., 1984b). Furthermore, a number of ATLA-positive non-human primate cell lines contain the same glycopolypeptide (M. Okada et al., unpublished). All of these cell lines were also positive for gp46, which was enriched by affinity chromatography from the virus- and cell-free culture medium (Yamamoto et al., 1982c). A
A tunicamycin experiment suggested that the non-glycosylated forms of gp68 and gp61 in MT-2 cells are p54 and p46, respectively (Koyanagi et al., 1984a). Thus, it is clear that the unglycosylated form of gp68 is distinct from that of gp61. Evidence that these glycoproteins are encoded by the viral genome was obtained using a rabbit antiserum against a synthetic decapeptide whose structure had been predicted from the nucleotide sequence of gp61 (Hattori et al., 1984) and immunoprecipitation by anti-gp68 rabbit serum of a p33 polypeptide after in vitro translation of viral RNA using a rabbit reticulocyte system (Kobayashi et al., 1984b). These data show that gp61 is an env precursor which is processed into gp46 and p15–20E. Takeuchi et al. (1985) have shown that gp68 is a read-through product containing parts of both the env and pX regions from the subgenomic 20S RNA in MT-2 cells.

The reverse transcriptase of HTLV was first characterized by Rho et al. (1981). The enzyme has a molecular weight of about 95000 and prefers Mg$^{2+}$ as the divalent cation. HTLV reverse transcriptase can be distinguished immunologically by neutralization tests from those of known type C, B and D retroviruses.

Genome structure of the virus

Reitz et al. (1981) showed that a cDNA probe synthesized by the endogenous reverse transcriptase reaction hybridized with HTLV 70S RNA but not with 70S RNA prepared from 16 strains of known animal retroviruses. They also studied the DNA of a variety of normal and malignant human and animal tissues and cell lines including HUT-102 for sequence homology to HTLV cDNA. The only significant homology was found in HUT-102 cells, but none in any other tissue or cell tested. This indicated that HTLV sequences are acquired exogenously. Yoshida et al. (1982) prepared cDNA from ATLV and studied the DNA obtained from PBL of ATL patients. Analysis of the DNA by Southern blotting after EcoRI digestion demonstrated that five patients with ATL unequivocally possessed HTLV proviral DNA whereas three normal controls without anti-ATLA antibodies were negative. DNA samples from a large number of ATL patients have now been analysed (Yoshida et al., 1984; Seiki et al., 1984; Yamaguchi et al., 1984); the results show that without exception the DNA of all ATL patients yielded discrete positive bands. But each DNA preparation from fresh lymphocytes produced only a single band and the sizes varied in different patients after EcoRI digestion. When the DNA from leukocytes of healthy virus carriers or patients with non-ATL leukaemia but who had anti-ATLA antibodies were analysed, a smear rather than discrete bands was observed. These data indicate that (i) viral DNA is integrated into ATL cell DNA, (ii) ATL cells are composed of a cell population that had grown monoclonally after virus infection, and (iii) the integration site of the provirus occurs randomly in the host cell chromosome. Yoshida et al. (1982) also studied the presence of virus-specific RNA by Northern blotting and showed that the MT-1 and MT-2 cell lines contained virus-specific 35S RNA and 20S RNA. The level of these viral messages in the cells increased after IUdR treatment in proportion to the increase of ATL-positive cells in the cell population. N. Kobayashi et al. (unpublished) also showed that all ATL-positive cell lines tested contained both genomic 35S RNA and subgenomic 26S RNA as the predominant transcripts of the ATLV genome.

Seiki et al. (1982) first reported the cloning and sequencing of proviral DNA containing the long terminal repeat (LTR) region. Subsequently, they succeeded in cloning the full-length proviral genome integrated in fresh leukaemic cell DNA into a Charon 4A vector (Seiki et al., 1983). The provirus was subcloned into pBR322 in several fragments and the nucleotide sequences of each were determined. The proviral genome of HTLV is composed of 9032 nucleotides. This sequence contains three large open reading frames coding for proteins, assigned as gag, pol and env in that order from the 5' end of the viral genome. The molecular weights of the translation products of these genes were calculated from the nucleotide sequence to be 48K, 99K and 54K respectively.

The 48K precursor was predicted to be processed into 14K, 24K and 9K polypeptides. Antibodies raised against synthetic peptides corresponding to parts of these three proteins demonstrated that polypeptides 14K, 24K and 9K corresponded to p19, p25 and p15, respectively (Hattori et al., 1984). The env gene product was also identified in a similar manner.
as being gp62, which was then processed into gp46 and p20E (Hattori et al., 1984). Interestingly, the sequence contains a fourth coding region downstream of env, termed the pX region. This sequence could code for four possible proteins of 10K to 27K. Contrary to expectation, this region did not contain a typical transforming gene because it was not homologous to the protoonc genes in normal cellular DNA (Seiki et al., 1983). Recently, this region was reported to be homologous to the genomic DNA of normal rat and mouse, indicating the possible derivation of the pX sequence from rodents (Fukui et al., 1984). The proviral genome of HTLV/ATLV and the proteins identified are shown in Fig. 3. All these data show that HTLV/ATLV is a replication-competent virus. In some cases, however, defective proviruses have been found in fresh leukaemic cells (Yoshida, 1984; N. Kobayashi et al., unpublished). Such defective proviral genomes retained the pX and 3' LTR regions, indicating a crucial role of the pX region of the viral genome in the maintenance of the leukaemic state (see below).

**Monoclonal antibodies**

Anti-p19 antibodies were first obtained by the mouse hybridoma procedure as a HTLV-specific monoclonal antibody (Robert-Guroff et al., 1981; Tanaka et al., 1983). One such antibody (GIN 14) reacted only with the cytoplasm of 12 ATLA-positive cell lines but not with negative cell lines (Tanaka et al., 1983). An interesting observation with this antibody was that it reacted with the p28 polypeptide of ATLA in MT-2 cells in addition to p19. This antibody was very useful for various purposes, e.g. competition assays, study of the gag precursor polypeptide, or determination of ATLA-positive cells in primary or long-term cultures of lymphocytes containing Ig-positive cells. Since monoclonal antibodies bind to specific antigenic determinants they only cross-react with different antigens having similar structures. Anti-HTLV p19 was shown to react not only with HTLV-infected lymphocytes but also with the thymus tissue of seronegative humans and syncytiotrophoblast cells from normal human placenta (Robert-Guroff et al., 1981). Monoclonal antibodies against p24 (Palker et al., 1984; Fujii et al., 1984) and gp21 (Sugamura et al., 1984a) have also been reported. Anti-p24 antibody has been used to identify a precursor protein, p53 (Fujii et al., 1984). Besides these HTLV-specific antibodies several monoclonal antibodies which react with ATL cells have been developed. Uchiyama et al. (1981) used HUT-102 cells as an immunogen and obtained antibodies that specifically reacted with activated human T-cells (anti-Tac antibodies). The Tac antigens detectable with
this antibody are considered to be the TCGF receptor molecules on the cell surface (Leonard et al., 1982). Another class of anti-TCGF receptor antibodies has been obtained by Tanaka et al. (1984). Since all ATLA-positive cells contain Tac antigens and leukaemic cells from ATL patients are reported to express this antigen in vivo at the plasma membrane, the relationship between the expression of this antigen and the growth of leukaemic cells will be very important for understanding ATL leukaemogenesis (Uchiyama et al., 1984) (see below).

**Seroepidemiology**

** ATL and anti-ATLA antibody**

Almost all ATL patients were found to have anti-ATLV antibody, when screened using the immunofluorescence technique with MT-1 cells (Hi numa et al., 1981, 1982b; Shimoyama et al., 1982). Up to now, more than 500 patients have been studied and only rarely have they been negative for antibodies. Thus, it is clear that this antibody is strongly associated with ATL but not other types of leukaemia or lymphoma. When the place of birth of the ATLA antibody-positive ATL patients was ascertained, many were found to have been born in Okinawa, Nagasaki and Kagoshima, and also to a lesser extent in Kumamoto, Shikoku and the southern part of the Kii Peninsula. Patients were frequently clustered in coastal areas, isolated islands or peninsulas. Occupationally, patients were frequently workers in primary industries such as farming, forestry and fishing. Large-scale screening of antibody in several parts of Japan revealed the important fact that there were many healthy seropositive donors in the endemic areas of ATL, in apparent correlation with the frequency of its occurrence (Hi numa et al., 1982b; Maeda et al., 1984). For example, a nation-wide survey of anti-ATLA in volunteer blood donors (16 to 64 years of age) indicated that the highest rate (3%) of seropositive donors occurred in Kyushu district and lower rates of 0.08 to 0.3% in all other districts. The frequency of seropositive donors increased with age. The seropositive rate varied considerably in different localities, even within districts with a high or low seropositive incidence. These healthy donors with serum antibody were subsequently found to be virus carriers, because they had ATLV-carrying lymphocytes in the peripheral blood as revealed by short-term cultivation (Hi numa et al., 1982a; Gotoh et al., 1982) or by Southern blotting analysis (Yoshida et al., 1982). Thus, it is obvious that ATL endemic areas also correspond to ATLV endemic areas. From these data it is apparent that ATL occurred only in a minor fraction of individuals infected with ATLV. Indeed, Kinoshita et al. (1982) found two ATL patients whose sera had been demonstrated to be anti-ATLA antibody-positive several years before the onset of ATL. As regards genetic predisposition, Tajima et al. (1984) could not regularly find a significant correlation between anti-ATLA antibodies and certain HLA phenotypes.

**Transmission of HTLV/ATLV**

Tajima & Hi numa (1984) reported a sexual difference in the prevalence of anti-ATLA antibody among healthy donors. There appeared to be no significant difference between the sexes up to 40 years of age, but then the prevalence of antibody in women increased with age more rapidly than in men. Moreover, seropositives were seen as clusters in the families of ATL patients (Miyoshi et al., 1982a; Tajima et al., 1984). Therefore, it is likely that ATLV is transmitted between members of the family of ATL patients. But this fact also indicates that the virus is not as infectious as other viruses such as EBV, influenza virus or poliovirus.

Several possible routes of transmission of ATLV have been considered. Vertical genetic transmission of infection is possible since animal retroviruses are commonly endogenous. However, this has been completely ruled out in the case of HTLV/ATLV (Reitz et al., 1981; Yoshida et al., 1982). Another possibility is that the virus can be transmitted by transplacental or intracervical infection, because familial studies indicated that transmission from mother to child might occur (Tajima et al., 1982). There is, however, little evidence for infection of infants since anti-ATLA antibodies are only rarely detectable in them. Another type of vertical transmission is via the mother's milk. This form of infection has been shown to occur in viral bovine leukaemia which to some extent resembles ATL (Miller, 1979; Ferrer et al., 1981).
Some animal retroviruses are also transmitted via infectious virus-containing milk (Law, 1962). In this regard, Kinoshita et al. (1984) and Nakano et al. (1984) reported that expression of ATLA could be demonstrated in phytohaemagglutinin-stimulated mononuclear cells of milk samples from seropositive mothers after delivery. Blood transfusion is a very important route of horizontal transmission of ATLV, as it is also the case of hepatitis B virus. From retrospective analysis, Okochi et al. (1984) demonstrated that 26 out of 41 recipients of whole blood or blood components containing cells from donors with anti-ATLA antibody, produced antibody. Interestingly no anti-ATLA was detected in all 14 recipients of fresh-frozen plasmas prepared from antibody-positive donors. None of 252 recipients of blood products containing cell components from anti-ATLA-negative donors produced anti-ATLA. IgM antibody was demonstrated in recipients who developed anti-ATLA antibody. Thus, primary infection is strongly associated with cells in blood from healthy virus carriers. The fact that only the patients who received whole blood or blood products containing cells, as opposed to cell-free plasma, developed ATLA antibody is in accord with the observation that transformation of normal lymphocytes occurs only as a result of co-culture in vitro which requires cell-to-cell contact. A possibility that vectors such as blood-sucking insects, e.g. mosquitoes and fleas, are involved in transmission of ATLV can not be excluded (Tajima et al., 1982, 1984). This has been suggested in the case of BLV transmission (Bech-Nielsen et al., 1978). In an ATLV endemic area (Okinawa), a high rate of ATLV antibody-positivity was observed in carriers of a parasite, Strongyloides stercoralis, in contrast to a lower rate in parasite-free controls. This suggests that parasite infection over a long period may promote ATLV infection or growth of the virus in vivo, resulting in the development of ATLV antibody (Nakada et al., 1984). The association between ATLV infection (and/or adult T-cell leukaemia) and S. stercoralis infection is comparable with the relation between filarial infection and ATLV noted in other ATL endemic areas (Tajima et al., 1983). Another important possible route of transmission revealed by seroepidemiological studies was between spouses, especially from husband to wife. Indeed, very recently Nakano et al. (1984) reported that mononuclear cells in semen from one of three anti-ATLA positive men tested were ATLA-positive. It has still to be determined whether HTLV/ATLV is transmitted by sexual contact as has been shown for hepatitis B virus. To study routes of HTLV transmission, it will be essential to develop a simple and rapid method for virus detection besides antibody screening.

ATL and anti-HTLV antibodies outside Japan

HTLV was first found and isolated from two black people living in the U.S.A. (Poiesz et al., 1980, 1981). In 1982 Catovsky et al. reported six ATL patients who had emigrated from the West Indies and Guyana to Great Britain. Later, anti-HTLV-positive ATL patients were also found in Jamaica (Blattner et al., 1983), Martinique (Gessain et al., 1984), south-eastern U.S.A. (Blayney et al., 1983a) and Surinam (Vyth-Dreese et al., 1983). These data indicated that the Caribbean basin is an ATL endemic area. In this region about 3% of healthy adults were infected with HTLV (Schupbach et al., 1983). However, an age-dependent increase in the prevalence of antibody was not observed in contrast to the situation in Japan (Hinuma et al., 1982b). Although these initial studies of ATL outside Japan showed that the Caribbean basin is a second ATL endemic area, further studies disclosed that ATL patients were sporadically found in other areas such as Ecuador, Israel, Brazil and Alaska (Blayney et al., 1983b). In Taiwan, sporadic cases of ATL were found, and about 1% of the Chinese population were estimated to be ATL carriers (Hinuma et al., 1983). In contrast, essentially no virus-carriers were found in mainland China (Zeng et al., 1984).

Discoveries of antibodies which cross-reacted with HTLV in African people and African non-human primates led Hunsmann et al. (1983) and Yamamoto et al. (1983a) to hypothesize that HTLV in the Caribbean basin might have originated from Africa. From seroepidemiological surveys of African people, it was found that 2% and 3-5% of blood donors among students in Kenya and Nigeria, respectively, were seropositive. Moreover, Fleming et al. (1983) reported that lymphoma patients with anti-HTLV antibodies existed in Nigeria. Hunsmann et al. (1984) extended these studies and showed that 1 to 6% of the population of several African countries, Senegal, Liberia, Nigeria, Kenya, Gabon, Zaire and South Africa, were infected with HTLV.
Although more extensive studies are required, this suggests that the African continent could be the largest HTLV endemic area. However, no evidence has been presented to suggest a relationship between Japanese and African HTLV/ATLV carriers.

**HTLV/ATLV strains**

In 1982, a new subtype of HTLV, called HTLV-II, which was related to but distinct from the prototype HTLV-I was reported (Kalyanaraman et al., 1982). This virus was derived from a cell line established from the spleen of a patient (MO) with a T-cell type of hairy cell leukaemia. HTLV-II shares several biological activities with HTLV-I. For example, it is able to infect both normal human T- and B-cells by co-cultivation methods, but transformation occurs in T-cells exclusively (Chen et al., 1983). The nucleotide sequence homology of the two viruses has been investigated. Studies carried out with Southern blotting and heteroduplex mapping at different melting points showed that both viruses had strongly conserved nucleotide sequences in the pX region and less-conserved regions in the LTR, gag, pol and env regions although they are still substantially homologous (Shaw et al., 1984). These data show that both viruses are evolutionarily divergent members of HTLV and suggest that the pX region of HTLV may play an important role in the transformation of cells. Transformation and replication of HTLV-II appear to occur most readily in T-cells, usually those having the OKT-4⁺ phenotype. Chen et al. (1984) studied the T-cell tropism of this virus using recombinants of LTR and a selectable gene (neomycin resistance) and indicated that restriction of expression is governed by cis-acting functions of the LTR. The aetiological role of HTLV-II in hairy cell leukaemia is not obvious since this virus has been found in only one patient (MO) with this disease.

Acquired immune deficiency syndrome (AIDS) was first reported in American homosexuals in 1981 (Centers for Disease Control, 1981). In 1983, Barre-Sinoussi et al. in France isolated a retrovirus from a patient with AIDS and named it lymphadenopathy-associated virus (LAV). In 1984, Gallo and his co-workers isolated a new type of retrovirus called HTLV-III from blood of AIDS patients (Gallo et al., 1984). Although no comparative studies have been reported, the two viruses are closely related if not identical (Popovic et al., 1984; Klatzmann et al., 1984; Kalyanaraman et al., 1984; Schupbach et al., 1984). These viruses resembled HTLV-I in some respects, particularly in biological activities. For example, the target cell of both viruses is a subset of T-cells with the OKT-4⁺ surface marker and their reverse transcriptases prefer Mg²⁺ over Mn²⁺ for maximal activity. LAV/HTLV-III may be the causative agent of AIDS for several reasons. (i) The virus is found in AIDS or pre-AIDS patients at high frequency. (ii) The virus causes specific c.p.e in OKT-4⁺ cells. (iii) The virus is transmissible by blood transfusion. (iv) Seroepidemiological studies have shown that AIDS or pre-AIDS patients frequently possess serum antibody against this virus; antibodies were also found in a proportion of homosexual males, but were rare in normal healthy donors. (v) LAV/HTLV-III caused an AIDS-like disease in chimpanzees. Essex et al. (1983) reported that AIDS patients had antibody that reacted with the membrane antigens of HTLV-I-positive cells at high frequency. This, however, has not been confirmed since many groups have failed to show a significant relationship between AIDS patients and HTLV-I infection (Barre-Sinoussi et al., 1983; Tedder et al., 1984).

**HTLV/ATLV in non-human primates**

Miyoshi et al. (1982c) first demonstrated that Japanese macaques possessed antibodies that reacted with human HTLV-positive cells. C-type particles indistinguishable from human ATLV were also induced from lymphocyte cultures from these animals as in man. Nation-wide surveys of troops of wild Japanese monkeys revealed that a high incidence (20 to 30%) of anti-ATLV-positive monkeys were present in most troops, not only in endemic areas of human ATL, but also in non-endemic areas (Miyoshi et al., 1983a; Hayami et al., 1984a). Yamamoto et al. (1983a) found that a high frequency (about 70%) of African green monkeys imported into and kept in West Germany were infected with ATLV-like agents as shown by immunofluorescence and immunoprecipitation tests. Several other investigators extended these studies and showed that all seropositive monkeys were Old World monkeys, e.g. rhesus monkeys, crab-eating monkeys and Formosan monkeys (mainly macaques of Asian and African origin). On the other hand, no
antibody was detected in prosimians and New World monkeys (Hunsmann et al., 1983; Miyoshi et al., 1983a; Hayami et al., 1983, 1984a). Some baboons and apes originating from Africa were also positive for antiviral antibodies (Hayami et al., 1984a; Guo et al., 1984; Botha et al., unpublished).

As was observed in epidemiological studies of humans, the incidence of seropositive individuals in Japanese monkeys increased gradually with age and reached a maximum when they became adult (Ishida et al., 1983; Hayami et al., 1984a). However, familial clustering of seropositive individuals was not obvious. The latter may be explained by the fact that in Japanese monkeys the virus is disseminated widely within the troop, extending beyond individual families.

Continuous lymphoblastoid cell lines carrying ATLV or ATLV-like agents were established from the PBL of various species of monkeys in the presence or absence of TCGF or by co-culture (M. Okada et al., unpublished; M. Hayami et al., unpublished). These animals included Japanese monkeys, red-faced monkeys, Formosan monkeys, pig-tailed monkeys, bonnet monkeys, African green monkeys and chimpanzees. Most of them are positive for ATLA detectable using natural antibodies to ATLV in human and monkey sera or monoclonal antibodies to ATLV. These cell lines, however, contained the surface markers not only of T but also of B and non-T, non-B cells. Monkey ATLV-positive cells were also shown to immortalize normal human lymphocytes derived from PBL and CBL (Miyoshi et al., 1983a; Yamamoto et al., 1984b; M. Okada et al., unpublished). The core polypeptides of monkey ATLV have molecular weights very similar to each other and to ATLV. Viruses from Japanese macaques and African green monkey had serologically distinct envelope polypeptides and their core polypeptides had different isoelectric points (Yamamoto et al., 1984b, c). Guo et al. (1984) also showed that baboon HTLV was closely related to but distinguishable from human HTLV as revealed by restriction endonuclease cleavage patterns of the viral genome using site-specific ATLV probes. These results are concordant with seroepidemiological data that show clear-cut differences between the geographical distribution of seropositive simians and seropositive humans (Hayami et al., 1984a). Thus, it is very unlikely that direct transmission of HTLV occurs between simians and humans or vice versa.

Experimental infection of animals with HTLV/ATLV

MT-1 cells at subcultures 92 to 97 were implanted intraperitoneally into newborn hamsters (Miyoshi et al., 1979). Eight out of 10 animals developed tumours which could be passaged serially in animals. As already described, MT-2 cells immortalized lymphocytes from monkeys and rabbits by co-culture. Inoculation of such ATLV-containing cells into crab-eating monkeys (Yamamoto et al., 1984d) and rabbits (Miyoshi et al., 1985; N. Yamamoto et al., unpublished), either heterologously or homologously, consistently yielded evidence of virus infection in these animals, namely the production of anti-ATLA antibodies and detection of viral antigen in their lymphocytes. It was also consistently observed that these animals did not develop any clinical or haematological abnormalities. However, the situation was different when normal rat lymphocytes were used. Tateno et al. (1984) established ATLA-positive rat cell lines with T-cell markers, designated TARS-1 and TART-1 by co-cultivation with MT-2 cells following bromodeoxyuridine treatment. TART-1 and TARS-1 were transplantable into newborn syngeneic rats and nude mice which were eventually killed. From this result the authors concluded that HTLV not only immortalizes but also malignantly transforms rat T-cells in vitro. This observation may be important in considering the role of HTLV in ATL leukaemogenesis in relation to a multi-step carcinogenesis model (Yamamoto, 1984).

Successful infection of animals with cell-free ATLV was first reported by Yamamoto et al. (1984d). Three crab-eating monkeys without serum antibody to ATLA were inoculated with cell-free virus. ATLA appeared in peripheral lymphocytes from all three animals, and they all developed an antibody response to the virus-specific glycopolypeptides and viral core polypeptides. Up to 1 year after inoculation no animal developed any haematological or clinical abnormality.
Hypothetical views of ATL leukaemogenesis

From the analysis of viral structures, HTLV/ATLV is a replication-competent, lymphatic leukaemia (LLV)-type virus with no oncogene in the classical sense. Thus, the familiar model for a number of animal retroviruses where non-transforming LLV-type virus becomes transforming after acquiring a normal cellular oncogene by transduction is not applicable to this system. However, it is also true that defective proviral genomes are found in primary and cultured ATL cells (Yoshida et al., 1982; Kobayashi et al., 1984a). Furthermore, transcription of such defective provirus does occur in MT-2 cells. Kobayashi et al. (1984c) showed that a serine-specific phosphokinase activity was associated with p28, which was encoded by a 24S defective proviral genome. This 24S defective genome is composed of portions of the gag and pX genes. Although the kinase activities associated with many animal retroviruses are tyrosine-specific, recently the murine sarcoma virus oncogene, mos, has been shown to be expressed as a gag-mos fusion gene and its gene product p85 gag-mos was associated with serine or threonine protein kinase activity rather than a tyrosine-specific activity (Kloetzer et al., 1983).

The p28 polypeptide was detected only in MT-2 cells and most MT-2-derived T-cell lines. This situation is reminiscent of the gp68 polypeptide, which was detected only in MT-2 cells and not in unrelated cells. Glycoprotein 68 was shown to be a fusion protein which also contains the pX IV region of HTLV, by using antibody prepared against a synthetic peptide (Miwa et al., 1984) and from the nucleotide sequence (Takeuchi et al., 1985). These two polypeptides may have an important function in transformation, since only MT-2 cells expressed p28 and gp68 and this is the only cell line of several ATLA-positive cell lines which is capable of transformation by the cocultivation procedure.

HTLV-II is also able to transform human T-cells (Chen et al., 1983). Haseltine et al. (1984) showed that the 1557 nucleotide-long sequence at the 3' terminus of HTLV-II can be divided into a 5' region that is not conserved between type I and II viruses, and a 3', 1011 nucleotide-long region that is highly conserved. The latter corresponds precisely with a long open reading frame in both HTLV-I and II. The proteins that could be encoded by these open reading frames have a molecular weight of about 38K. A possibly important role of the pX region of HTLV was further proposed by Chen et al. (1984) for HTLV-II and Sodroski et al. (1984) for HTLV-I. They studied the influence of sequences within the LTR on gene expression using recombinant plasmids and obtained an indication that a factor can facilitate transcription from the viral promoter wherever this is located in the genome, which is consistent with the random integration of HTLV-I in the host cell chromosome. This type of regulation is called trans-acting transcriptional activation. Sodroski et al. (1984) suggested that a product of the 3' terminus of the pX gene itself acts as the trans-regulatory protein of LTR function. Several groups using sera from patients with ATL or from healthy carriers and antibodies against synthetic peptides identified 40K to 42K polypeptide in various ATLA-positive cell lines including MT-2 and HUT-102 cells as a product encoded by frame IV in the pX region of HTLV (Lee et al., 1984; Slamon et al., 1984; Kiyokawa et al., 1984; Miwa et al., 1984; Takeuchi et al., 1985). Because sera from patients with ATL contained antibodies against the 40K polypeptide it is likely that it is expressed in vivo and plays a crucial role in transformation.

Uchiyama et al. (1984) using anti-Tac monoclonal antibody studied the role of TCGF receptors on the cell surface in ATL leukaemogenesis. Their results are summarized as follows. (i) Primary or cultured leukaemic cells from ATL patients constitutively express TCGF receptors on the cell surface in ATL leukaemogenesis. Their results are summarized as follows. (ii) Although antigenic modulation, or down-regulation, of Tac antigen on activated normal T-cells is induced by anti-Tac antibody, the expression of Tac antigen on ATL cells or T-cell lines is not affected. (iii) Tac antigens of activated normal T cells are phosphorylated in response to the addition of TCGF whereas this occurs autonomously and constitutively in the case of MT-1 cells independently of the growth factor. Thus, TCGF on the surface of leukaemic cells from ATL patients appeared to be aberrantly expressed either in terms of response to exogenous growth factor or in terms of phosphorylation. Recently Sugamura et al. (1984c) detected expression of Tac antigen on EBV-transformed human B-cell
clones that were simultaneously infected with ATLV. Following this finding, they obtained direct evidence of TCGF receptor induction as a result of ATLV infection of human B-cell lines (Sugamura et al., 1984c). It remains to be determined how the abnormal expression of the TCGF receptor in ATL cells is related to HTLV/ATLV infection, particularly with regard to the role of the \( pX \) genome region and leukaemic expansion of ATL cells. Our preliminary studies show that a continuously growing cell line with T-helper phenotype and TCGF receptors was obtained in the presence of TCGF by transfection of a molecularly cloned \( \text{gag-}pX \) fragment into normal human lymphocytes (Y. Koyanagi et al., unpublished). At present, no data are available on whether the entire DNA genome of HTLV-I can be used for transformation and to obtain biologically active virus; the latter is certainly the case with HTLV-III (Chen et al., 1984).

**Overall conclusions**

Regarding the cause of leukaemias or malignant lymphomas, several advances in viral oncology and immunology have merged recently, resulting in a heightened appreciation of the associations between viral infection, immunodeficiency and genetic predisposition. In humans, EBV infection has been discussed thoroughly from these standpoints in relation to BL, X-linked lymphoproliferative syndrome, ataxia telangiectasia and malignant lymphomas after organ transplantations (Purtilo et al., 1984). In a previous section describing possible mechanisms of leukaemogenesis of ATL we discussed mainly transformation at the cellular level and not at the level of the host organism. It is apparent, however, that the fate of the transformed cell at the cellular level will be determined eventually by confrontation with the various immunological barriers of the host. Variation at the cellular level, of course, should be closely related to a changed response to the host immune mechanism. Cytogenetic alterations most probably endow transformed cells not only with a growth advantage at the cellular level but also with an ability to escape the immune surveillance of the host. The results described in this context clearly indicate that HTLV/ATLV is a prerequisite for the occurrence of ATL. It is also obvious, however, that this disease is 'a chronic malignancy' which requires a long period of time between viral infection and the occurrence of disease. This strongly indicates that some forces operate to select out a single cell and to initiate its monoclonal growth from the population of the cells which had been infected polyclonally with HTLV/ATLV. Although HTLV/ATLV is one of the major factors in the development of ATL, one or more additional factors seem to be necessary. Such factors can be assumed to be both exogenous and endogenous. It appears to us that ATL carcinogenesis results from the interaction of HTLV/ATLV with various biological, physical and chemical factors in the environment (Yamamoto, 1984).

Original studies by the authors were supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare, Japan, by the Deutsche Forschungsgemeinschaft and by the Alexander von Humboldt Stiftung.

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