JOURNAL OF VIROLOGY, Nov. 1990, p. 5682–5687
0022-538X/90/115682-06$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 64, No. 11

In Vivo Cellular Tropism of Human T-Cell Leukemia Virus Type 1

J. H. RICHARDSON,[1]†* A. J. EDWARDS,[2]‡ J. K. CRUICKSHANK,[3] P. RUDGE,[3] AND A. G. DALGLEISH[1]

Retrovirus Research Group,[1] Department of Transplantation Biology,[2] and Department of Medicine,[3] MRC Clinical Research Centre and Northwick Park Hospital, Harrow, Middlesex HA1 3UJ, United Kingdom

Received 26 April 1990/Accepted 7 August 1990

To establish the phenotype of human T-cell leukemia virus type 1 (HTLV-1)-infected cells in peripheral blood, the polymerase chain reaction was used to detect and quantitate viral DNA in subpopulations of leukocytes obtained from patients with tropical spastic paraparesis and asymptomatic carriers. HTLV-1 could not be detected in peripheral blood mononuclear cells thoroughly depleted of T lymphocytes (E− CD3−), nor could it be detected in highly enriched populations of B lymphocytes (E− CD19+), monocytes (E− CD14+), or natural killer cells (E− CD16+). T lymphocytes were strongly positive for HTLV-1, and fractionation of this population revealed that 90 to 99% of the HTLV-1 DNA segregated with the CD4+ CD8− and CD45RO+ subsets. No difference between the cell type distribution of HTLV-1 in the asymptomatic carrier and the subjects with tropical spastic paraparesis was evident. Southern hybridization of genomic DNA prepared from the peripheral blood of HTLV-1 carriers indicated that up to 10% of circulating leukocytes may carry the HTLV-1 provirus.

The retrovirus human T-cell leukemia virus type 1 (HTLV-1) is the causal agent of adult T-cell leukemia-lymphoma (13, 39) and has more recently been shown to be closely associated with the chronic neurodegenerative disorder tropical spastic paraparesis (TSP) (9), also known as HTLV-1-associated myelopathy. A knowledge of which cell types harbor the virus in infected individuals is clearly important for understanding the natural history of infection and may also provide clues to the pathogenesis of HTLV-1-associated disease.

HTLV-1 is generally considered to be T-lymphocyte tropic and to infect predominantly the helper T-cell subset defined by surface antigen CD4. This assumption is based on two observations. First, the leukemic cell in adult T-cell leukemia-lymphoma, which almost always carries an HTLV-1 provirus, is usually a CD4-positive (CD4+) T lymphocyte (2, 12). Second, CD4+ is the predominant phenotype of virus-carrying immortalized T-cell lines, which can be obtained either upon cultivation of peripheral blood mononuclear cells (PMBC) from HTLV-1 carriers (10, 27) or by cocultivation of uninfected adult or cord blood mononuclear cells with lethally irradiated HTLV-1-producing cells (22, 36). Such culture techniques have sometimes yielded HTLV-1-infected B-lymphocyte lines (19, 35, 38) and CD8+ T-cell lines (30, 36), but it has not been established whether these cells are targets for infection in vivo. Both cloned T-cell lines and bulk T-cell populations expressing CD8 are susceptible to experimental infection and immortalization by HTLV-1 (6, 20), but attempts to infect B lymphocytes in vitro have generally been unsuccessful (18, 30).

A number of nonlymphoid human cell types have been productively infected with HTLV-1 in vitro. These include primary endothelial (16) and glial (31) cells as well as several nonlymphoid tumor lines (1, 3, 14). Studies with vesicular stomatitis virus (HTLV) pseudotype viruses confirm a broad if not ubiquitous distribution of HTLV-1 receptor molecules on diverse tumor cell lines from a wide range of mammalian species (26). The potential of HTLV-1 to infect a range of cell types led us to examine in closer detail the phenotype of HTLV-1-carrying cells in the peripheral blood of infected individuals. In this study, we used the polymerase chain reaction (PCR) to detect and quantitate HTLV-1 DNA in fractionated peripheral blood leukocytes obtained from asymptomatic HTLV-1 carriers and patients with TSP. All studies were performed on fresh, uncultured material to avoid artifacts (such as secondary infection) that might arise from mitogen stimulation and in vitro cultivation.

Heparinized venous blood was obtained from five TSP patients (one on two occasions) and one healthy virus carrier whose HTLV-1 antibody titer was comparable with that of the TSP patients. Blood was mixed with 0.3 volumes of Dextraven (Fisons) and left undisturbed for 30 min to allow erythrocytes to settle. The supernatant was separated on a Ficoll-Paque density gradient to yield a mononuclear cell-enriched layer at the Ficoll-plasma interface and a polymorphonuclear granulocyte-enriched pellet. The granulocytes were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum after hypotonic lysis of residual erythrocytes. Mononuclear cells from the interface were rosetted with sheep erythrocytes to obtain E-rosette-positive (E+; T-cell) and E-rosette-negative (E−; non-T-cell) populations and suspended in RPMI 1640–10% fetal calf serum. E− cells were further depleted of T cells and fractionated into phenotypic subsets, using a fluorescence-activated cell sorter (Becton Dickenson FACSTAR PLUS) according to one of the following procedures. (i) E− cells were labeled with Leu 11a-fluorescein isothiocyanate (FITC) and Leu 12-phycoerythrin (PE); CD19+ (B-lymphocyte) and CD16+ (natural killer cell) populations were recovered. (ii) E− cells were labeled with Leu 4-FITC and Leu M3-PE; CD3− CD14+ (monocyte) and CD3− CD14− (all other non-T-cell) populations were recovered. (iii) E− cells were labeled with Leu 4-FITC, and all CD3− (non-T) cells were collected. The E+ population was similarly divided into subsets by using flow cytometry. CD4+ CD8− and CD8+ CD4− populations were collected after labeling of cells with Leu 3a-FITC and Leu 2a-PE. E+ cells were also indirectly labeled by using

* Corresponding author.
† Present address: Department of Communicable Diseases, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, United Kingdom.
‡ Present address: FACS Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom.

TABLE 1. Antigens and MAb used to define mononuclear cell populations

Cell type	Cell surface antigen	MAb
T lymphocyte	CD3	Leu 4
B lymphocyte	CD19	Leu 12
Natural killer cell	CD16	Leu 11a
Monocyte	CD14	Leu M3
MHC[a] class II-restricted T cell	CD4	Leu 3a
MHC class I-restricted T cell	CD8	Leu 2a
Memory T cell	CD45RO	UCHL1

[a] MHC, Major histocompatibility complex.

the anti-CD45RO monoclonal antibody (MAb) UCHL1, followed by $F(ab')_2$ rabbit antimouse immunoglobulin-FITC (Dako); cells exhibiting highest and lowest fluorescence intensities were recovered. The MAb used to define mononuclear cell populations are shown in Table 1. All MAb were purchased from Becton Dickinson with the exception of UCHL1, which was a gift from P. Beverley (University College, London).

Cell samples were prepared for PCR analysis by boiling for 15 min in distilled water at 10^7 cells per ml. Lysates were clarified by centrifugation for 5 min at 13,000 × g, and 10-μl samples representing 10^5 cells were subjected to PCR, using primers to the pX region of the HTLV-1 genome. These primers amplify a 235-bp section and have previously been shown to detect fewer than 10 copies of HTLV-1 in a reproducible manner (29). The PCR reaction conditions, primers, probe, and hybridization protocol have been described elsewhere (29). Briefly, samples were given 35 to 40 cycles of amplification (60°C for 0.1 min, 72°C for 0.5 min, and 93°C for 0.1 min). One-fifth of the reaction mixture was run on a 2.5% agarose gel, transferred to a Zetaprobe membrane, and probed with a ^{32}P-labeled oligonucleotide. Titration of HTLV-1 DNA in samples was achieved by serial 10-fold dilution of cell lysates before PCR analysis.

The results of PCR analysis performed on fractionated leukocyte populations are summarized in Table 2 and Fig. 1. Non-T-cell populations obtained by E rosetting alone contained a significant proportion of T cells (1 to 5%, as determined by flow cytometry) and were invariably positive for HTLV-1 by PCR (not shown). In five of the six cases, non-T-cell populations devoid of HTLV-1 sequences were obtained after a further round of T-cell depletion, using a flow cytometer as indicated above. Entire non-T-cell populations as well as individual B-cell, natural killer cell, and monocyte preparations obtained in this manner were free of detectable HTLV-1 DNA (Fig. 1; Table 2). HTLV-1 DNA was detected in the flow cytometer-enriched non-T-cell fraction from patient 5 at the highest sample concentration. This result may be significant but equally may illustrate the limitations of the cell separation procedures used.

The E+ fraction, comprising 85 to 95% CD3+ (T) cells and 5 to 15% CD16+ (natural killer) cells, was clearly the predominant virus-infected population in peripheral blood (Fig. 1A). In four subjects, flow cytometry was used to separate this population into CD4+ and CD8+ subsets and also into cells positive and negative for CD45RO (also called UCHL1), the 180-kDa isoform of CD45. Cells expressing the CD45RO antigen have the functional characteristics of previously activated or memory T cells (34), whereas those that do not and express instead the CD45RA (CD45R, 2H4) isoform may represent naive or unstimulated cells (reviewed in reference 32). Initial PCR analysis indicated that the viral DNA was primarily associated with the CD4+ and CD45RO+ subsets (Fig. 1B). Limiting-dilution PCR analysis confirmed that these subsets contained between 10- and 100-fold more viral DNA than did the reciprocal CD8+ and CD45RO− populations (Fig. 1B and C; Table 2). The 1 to 10% of viral DNA that was associated with the CD8+ and CD45RO− populations may be partially attributable to residual CD4+ and CD45RO+ cells, respectively.

Polymorphonuclear granulocyte fractions were positive for HTLV-1 in five of the six cases (Table 2). The PCR-positive preparations did not contain T cells, as determined by flow cytometry or cytochemical staining with Leu 4 using the alkaline phosphatase–anti-alkaline phosphatase technique (24). A low level of contamination with infected T lymphocytes is likely, however, since only a single Ficoll-Paque centrifugation step was used to separate the mononuclear and polymorphonuclear populations.

The frequency of HTLV-1-infected cells in peripheral blood can be estimated from the minimum number of cells required to produce a positive PCR result (that is, which contain 10 or fewer viral genomes [29]). The minimum number of CD4+ and CD45RO+ cells was found to lie between 10 and 100 (Table 2). These data indicate that minimally 1% and possibly a much higher proportion of circulating CD4+ and CD45RO+ lymphocytes were infected, assuming that infected cells contain one or very few proviruses (39).

Southern blot analysis of genomic DNA prepared from whole peripheral blood of HTLV-1 carriers provided an

TABLE 2. PCR titration of HTLV-1 DNA in purified cell populations

Patient	Determination for given phenotype[a]						
	PMNG	E−[b]	E+	CD4+	CD8+	CD45RO+	CD45RO−
1	−		+				
2	10,000	− (i)	100				
3[c]	1,000	− (i)	10				
3a	1,000	− (ii)		100	10,000	100	1,000
4[d]	10,000	− (iii)		100	1,000	100	1,000
5	1,000	100,000 (iii)		100	1,000	100	1,000
6	10,000	− (iii)		100	10,000	100	10,000

[a] Number of cells in the greatest serial dilution to give a positive PCR result. +, Sample was positive with use of 10^5 cells but was not titrated; −, negative result with use of 10^5 cells. PMNG, Polymorphonuclear granulocytes.
[b] (i), (ii), and (iii) refer to cell separation protocols described in the text.
[c] Blood was obtained on two occasions.
[d] Healthy HTLV-1 carrier.

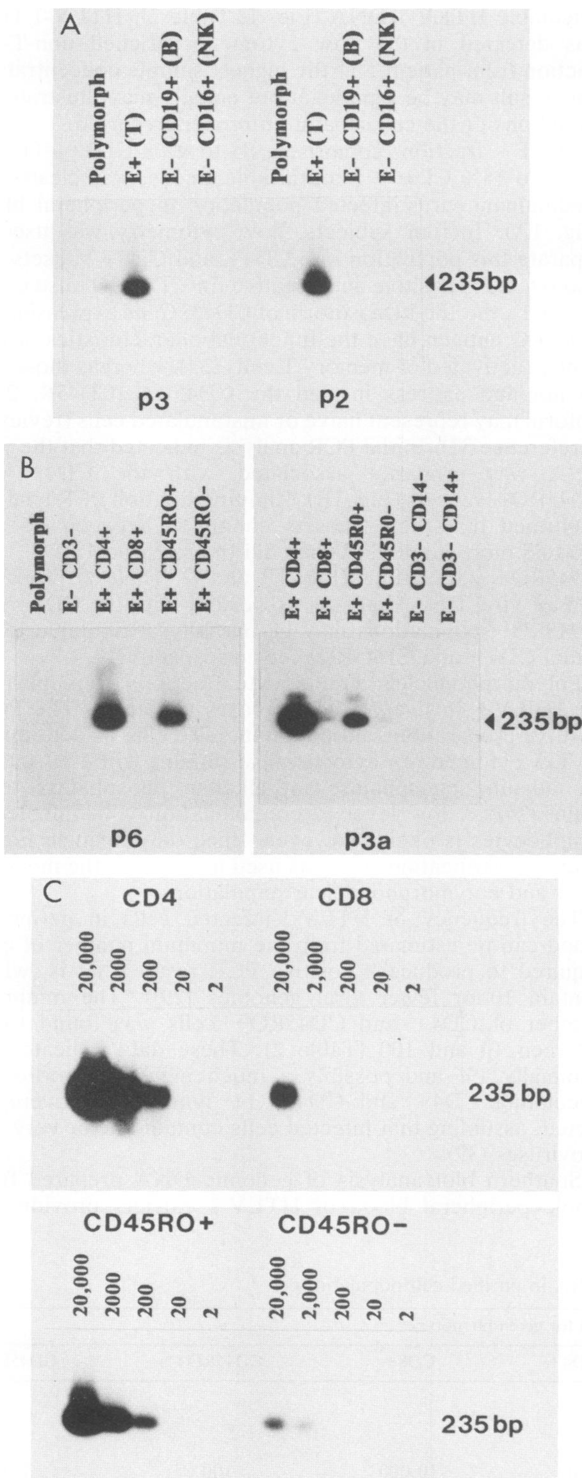

FIG. 1. (A and B) PCR analysis of fractionated cell populations from patients 2, 3, 3a, and 6, using 10^5 cell equivalents per reaction. (C) Limiting-dilution PCR analysis of T-cell subsets from patient 3a. The number of cell equivalents per reaction is indicated above each lane.

independent means of measuring virus load. Figure 2A shows a Southern blot in which peripheral blood DNA from asymptomatic carriers and TSP patients was digested with *Pst*I and probed with an HTLV-1-containing plasmid, pMT-2 (Fig. 2B). HTLV-1 DNA was detectable in DNA from 9 of 10 TSP patients and 4 of 6 asymptomatic carriers. From a series of plasmid copy number controls (Fig. 2A, lanes a to f), it could be deduced that the provirus load in the nine positive TSP patients (lanes m to v) ranged from 0.03 to 0.3 per cell and was typically 0.1 per cell. This finding suggests that in most carriers with TSP, about 10% of leukocytes are infected. A lower provirus load was found in the asymptomatic group (lanes g to l). *Pst*I digestion of the genomic DNA samples yielded the expected three internal restriction fragments of HTLV-1, but chromosomal junction fragments were not evident, indicating lack of clonality in the infected-cell population. The estimated 10% frequency of infected leukocytes in TSP patients is consistent with the proportion of PBMC that are found to express HTLV-1 antigen after short-term culture in vitro (5).

Although the number of HTLV-1 carriers investigated here is small, a consistent pattern of virus distribution in different leukocyte populations was found. In all subjects, the virus was predominantly associated with CD4+ lymphocytes and in addition was restricted to T cells expressing the CD45RO antigen. CD45RO is present on about 70% of peripheral CD4+ T cells and defines a subset of these cells containing a high proportion of the recall antigen-reactive cells which are thought to represent memory cells (34). Interestingly, this T-cell subset is reported to be overrepresented in Japanese patients with HAM (17, 25).

The basis of the target cell restriction exhibited by HTLV-1 has not yet been established. Receptor distribution is the key determinant of cell tropism for some retroviruses, including human immunodeficiency virus (21). A vesicular stomatitis virus (HTLV) pseudotype virus assay has shown that receptors for HTLV-1 are widely distributed on tumor cell lines (26), but studies of primary cells are few (18). One analysis of PBMC suggested that viral receptors were less abundant on non-T cells than on T cells (18), and this factor may contribute to the lack of infection of non-T cells in vivo. Target cell restriction can operate at a number of levels other than the receptor, however, and further studies are needed to establish the molecular basis of the cellular tropism displayed by HTLV-1 in vivo.

The finding that blood monocytes are not infected in our group of TSP patients is notable in view of the similar pathologies (4) of TSP and visna, a chronic neurological disease of sheep that is associated with infection by a monocyte-tropic retrovirus (8). Another retrovirus that causes neurological disease in humans, human immunodeficiency virus type 1, has also been postulated to gain access to the central nervous system in cells of the monocyte-macrophage lineage (15). Data presented here suggest that the same route does not operate in HTLV-1 infection.

The predominant association of HTLV-1 with the CD45RO+ subset of T cells thought to represent previously activated or memory cells (32, 34) is consistent with the recognized role of cell activation in retrovirus infection. In vitro studies have shown that resting cells are not permissive for productive infection by human or animal retroviruses (37, 40), apparently because of a block at the level of proviral DNA synthesis or integration that can be overcome if the cells are stimulated after exposure to the virus. A plausible explanation for our CD45RO data is that T lymphocytes can be infected by HTLV-1 regardless of their state of matura-

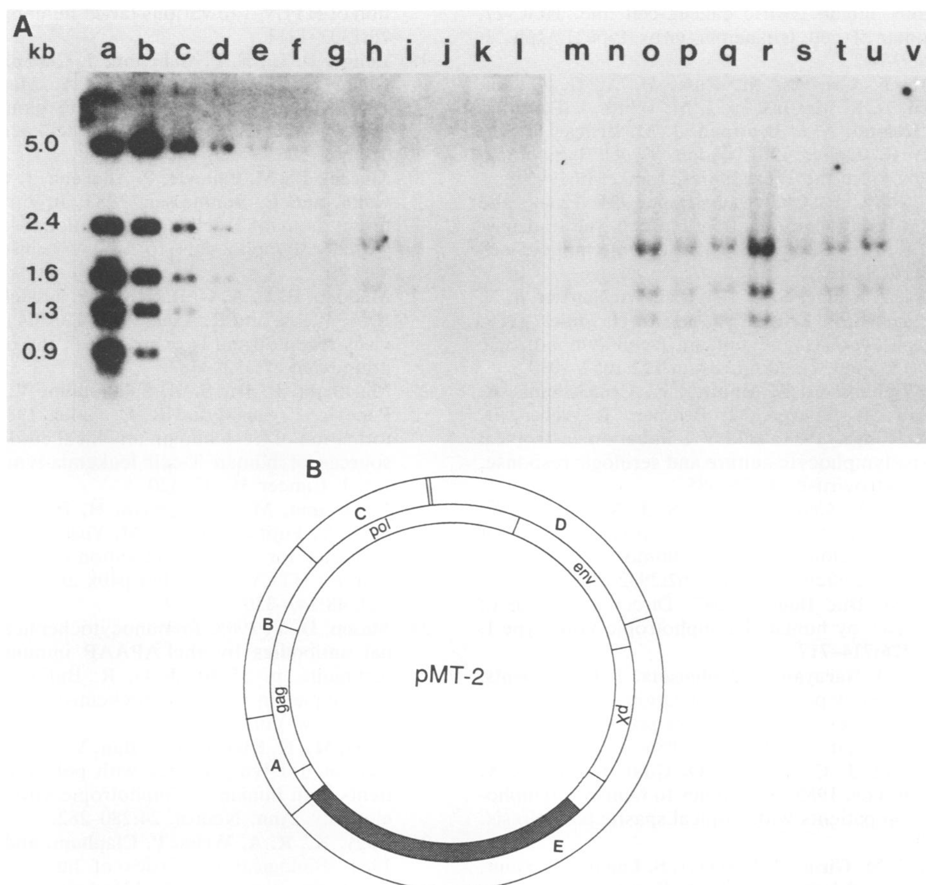

FIG. 2. (A) Southern blot analysis of DNA from HTLV-1 carriers. A 10-μg sample of DNA was digested with *Pst*I and probed with plasmid pMT-2. Lanes: a to f, 18, 6, 0.6, 0.18, and 0.06 pg of pMT-2 digested with *Pst*I (equivalent to 3, 1, 0.3, 0.1, 0.03, and 0.01 copies per diploid genome); g to l, asymptomatic HTLV-1 carrier DNA; m to v, TSP patient DNA. (B) Diagram of pMT-2 indicating positions of viral genes (inner band) and *Pst*I fragments (outer band). *Pst*I fragment lengths are 0.95 (A), 1.64 (B), 1.28 (C), 2.42 (D), and 5.0 (E) kb. Filled box represents cloning vector pSP65.

tion, but unless the cells are subsequently activated (thereby becoming positive for CD45RO), the infection will be abortive. Activation of the cell after exposure to HTLV-1 may be not only a prerequisite for successful infection but also a consequence of it. HTLV-1 is known to induce the expression of interleukin-2 and interleukin-2 receptor genes in infected T cells (23), thereby providing one possible mechanism of cellular activation. A second is suggested by reports that heat-inactivated HTLV-1 virions themselves have mitogenic properties in some circumstances (7).

A striking aspect of HTLV-1 infection to emerge from this work, also reported by Gessain and colleagues (11), is the high provirus load in peripheral blood. Southern blot analysis of genomic DNA prepared from whole peripheral blood suggested that approximately 10% of all leukocytes carry an HTLV-1 provirus in subjects with TSP, assuming that infected cells contain a single provirus (39). The proportion of infected leukocytes appeared to be slightly lower in asymptomatic carriers but ranged from less than 3% up to 10%. As we have shown that HTLV-1 is largely confined to CD4+ T cells, which constitute at most 30% of leukocytes in whole peripheral blood, the frequency of infected CD4+ T cells in TSP patients and some healthy carriers may be as high as one in three. Independent corroboration of both the high frequency and CD4+ phenotype of infected lymphocytes in HTLV-1 carriers has been obtained by the analysis of randomly generated T-cell clones from patients with TSP (K. W. Wucherpfennig, J. H. Richardson, and D. A. Hafler, unpublished observations). The high provirus load in TSP patients is in marked contrast to the situation in human immunodeficiency virus-infected individuals, in whom a much smaller proportion (0.01 to 1%) of PBMC harbor the virus (28, 33), and emphasizes the lack of cytopathic effect associated with HTLV-1 infection. This high virus burden may account for the very potent humoral immune response to HTLV-1 found in TSP patients (5).

This study shows that CD4+ T lymphocytes are the predominant leukocytes to be infected by HTLV-1 in vivo. In view of the ease with which purified CD8+ T lymphocytes can be infected in vitro, (6, 20), the basis of the preferential infection of CD4+ cells both in vivo and in vitro remains to be elucidated. Data presented here further suggest a critical role for lymphocyte activation in the establishment of infection in vivo.

This work was supported by a grant from the Multiple Sclerosis Society of Great Britain and Northern Ireland.

We thank P. Beverley for the gift of MAb UCHL1 and R. Gallo for providing plasmid pMT-2.

LITERATURE CITED

1. **Akagi, T., T. Yoshino, M. Motoi, H. Takata, S. Yano, I. Miyoshi, T. Oka, and Y. Ohtsuki.** 1988. Isolation of virus-producing

transformants from human gastric cancer cell line, HGC-27, infected with human T-cell leukaemia virus type I. Jpn. J. Cancer Res. 79:836–842.

2. **Catovsky, D., M. F. Greaves, M. Rose, D. A. G. Galton, A. W. G. Goolden, D. R. McCluskey, J. M. White, I. Lampert, G. Bourikas, R. Ireland, A. I. Brownell, J. M. Bridges, W. A. Blattner, and R. C. Gallo.** 1982. Adult T cell lymphoma-leukaemia in blacks from the West Indies. Lancet i:639–643.
3. **Clapham, P., K. Nagy, R. Cheingsong-Popov, M. Exley, and R. A. Weiss.** 1983. Productive infection and cell-free transmission of human T-cell leukaemia virus in a non-lymphoid cell line. Science 222:1125–1127.
4. **Cruickshank, J. K., P. Rudge, A. G. Dalgleish, M. Newton, B. N. McLean, R. O. Barnard, B. E. Kendall, and D. H. Miller.** 1989. Tropical spastic paraparesis and human T-cell lymphotropic virus type 1 in the United Kingdom. Brain 112:1057–1090.
5. **Dalgleish, A., J. Richardson, E. Matutes, K. Cruickshank, A. Newell, A. Sinclair, R. Thorpe, M. Brasher, J. Weber, D. Catovsky, and P. Rudge.** 1988. HTLV-1 infection in tropical spastic paraparesis: lymphocyte culture and serologic response. AIDS Res. Hum. Retroviruses 4:475–485.
6. **Faller, D. V., M. A. V. Crimmins, and S. J. Mentzer.** 1988. Human T-cell leukaemia virus type I infection of CD4+ or CD8+ cytotoxic T-cell clones results in immortalization with retention of antigen specificity. J. Virol. 62:2942–2950.
7. **Gazzolo, L., and M. Duc Dodon.** 1987. Direct activation of resting T lymphocytes by human T-lymphotropic virus type I. Nature (London) 326:714–717.
8. **Gendelman, H. E., O. Narayan, S. Molineaux, J. E. Clements, and Z. Ghobti.** 1985. Slow persistent replication of lentiviruses: role of tissue macrophages and macrophage precursors in bone marrow. Proc. Natl. Acad. Sci. USA 82:7086–7090.
9. **Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, A. Calender, and G. de The.** 1985. Antibodies to human T lymphotropic virus type 1 in patients with tropical spastic paraparesis. Lancet ii:407–410.
10. **Gessain, A., F. Saal, M. Giron, J. Lasneret, S. Lagaye, O. Gout, G. de The, F. Sigaux, and J. Peries.** 1990. Cell surface phenotype and human T lymphotropic virus type 1 antigen expression in 12 T cell lines derived from peripheral blood and cerebrospinal fluid of West Indian, Guayanese and African patients with tropical spastic paraparesis. J. Gen. Virol. 71:333–341.
11. **Gessain, A., F. Saal, O. Gout, M. Daniel, G. Fladrin, G. de The, J. Peries, and F. Sigaux.** 1990. High human T-cell lymphotropic virus type I proviral DNA load with polyclonal integration in peripheral blood mononuclear cells of French West Indian, Guianese, and African patients with tropical spastic paraparesis. Blood 75:428–433.
12. **Hattori, T., T. Uchiyama, T. Toibana, K. Takatsuki, and H. Uchino.** 1981. Surface phenotype of Japanese adult T cell leukaemia cells characterised by monoclonal antibodies. Blood 58:645–647.
13. **Hinuma, Y., K. Nagata, M. Hanaoka, M. Misoka, M. Nakal, T. Matsumoto, K. Kinoshita, S. Shirakawa, and I. Miyoshi.** 1981. Adult T cell leukaumia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc. Natl. Acad. Sci. USA 78:6476–6480.
14. **Hiramatsu, K., M. Masuda, and H. Yoshikura.** 1986. Mode of transmission of human T cell leukaemia virus type I (HTLV-1) in a human promyelocytic leukaemia HL60 cell. Int. J. Cancer 37:601–606.
15. **Ho, D. D., R. J. Pomerantz, and J. C. Kaplan.** 1987. Pathogenesis of infection with human immunodeficiency virus. N. Engl. J. Med. 317:278–286.
16. **Hoxie, J. A., D. M. Matthews, and D. B. Cines.** 1984. Infection of human endothelial cells by human T-cell leukaemia virus type I. Proc. Natl. Acad. Sci. USA 81:7591–7595.
17. **Itoyama, Y., S. Minato, J. Kira, I. Goto, H. Sato, K. Okochi, and N. Yamamoto.** 1988. Altered subsets of peripheral blood lymphocytes in patients with HTLV-1 associated myclopathy (HAM). Neurology 38:816–818.
18. **Krichbaum-Stenger, K., B. J. Poiesz, P. Keller, G. Ehrlich, J. Gavalchin, B. H. Davis, and J. L. Moore.** 1987. Specific adsorption of HTLV-1 to various target human and animal cells. Blood 70:1303–1311.
19. **Longo, D. L., E. P. Gelmann, J. Cossman, R. A. Young, R. C. Gallo, S. J. O'Brien, and L. A. Matis.** 1984. Isolation of HTLV-transformed B-lymphocyte clone from a patient with HTLV associated adult T-cell leukaemia. Nature (London) 310:505–506.
20. **Macchi, B., M. Popovic, P. Allavena, J. Ortaldo, P. Rossi, R. C. Gallo, and E. Bonmassar.** 1987. In-vitro susceptibility of different human T-cell subpopulations and resistance of large granular lymphocytes to HTLV-1 infection. Int. J. Cancer 40:1–6.
21. **Maddon, P. J., A. G. Dalgleish, S. J. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell 47:333–348.
22. **Markham, P. D., S. Z. Salahuddin, V. S. Kalyanaraman, M. Popovic, P. Sarin, and R. C. Gallo.** 1983. Infection and transformation of fresh human umbilical cord blood cells by multiple sources of human T-cell leukemia-lymphoma virus (HTLV). Int. J. Cancer 31:413–420.
23. **Maruyama, M., H. Shibuya, H. Harada, M. Hatakeyama, M. Seiki, T. Fujita, J. Inoue, M. Yoshida, and T. Taniguchi.** 1987. Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-1 encoded p40x and T3/Ti complex triggering. Cell 48:343–350.
24. **Mason, D. Y.** 1985. Immunocytochemical labelling of monoclonal antibodies by the APAAP immunoalkaline phosphatase technique, p. 25–40. *In* G. R. Bullock and P. Petrusz (ed.), Techniques in immunocytochemistry, vol. 3. Academic Press, Inc., New York.
25. **Mori, M., K. Kinoshita, N. Ban, Y. Yamada, and H. Shiku.** 1988. Activated T-lymphocytes with polyclonal gammopathy in patients with human T-lymphotropic virus type I-associated myelopathy. Ann. Neurol. 24:280–282.
26. **Nagy, K., R. A. Weiss, P. Clapham, and R. Cheingsong-Popov.** 1984. Biological properties of human T-cell leukemia virus envelope antigens, p. 121–131. *In* R. C. Gallo, M. Essex, and L. Gross (ed.), Human T-cell leukemia/lymphoma viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Popovic, M., P. S. Sarin, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann, J. Minowada, and R. C. Gallo.** 1983. Isolation and transmission of human retrovirus (human T cell leukaemia virus). Science 219:856–859.
28. **Psallidopoulos, M. C., S. M. Schnittman, L. M. Thompson, M. Baseler, A. S. Fauci, H. C. Lane, and N. P. Salzman.** 1989. Integrated proviral human immunodeficiency virus type 1 is present in CD4+ and peripheral blood lymphocytes in healthy seropositive individuals. J. Virol. 63:4626–4631.
29. **Richardson, J. H., K. W. Wucherpfennig, N. Endo, P. Rudge, A. G. Dalgleish, and D. A. Hafler.** 1989. PCR analysis of DNA from multiple sclerosis patients for the presence of HTLV-1. Science 246:821–823.
30. **Ruscetti, F. W., M. Robert-Guroff, L. Ceccherini-Nelli, J. Minowada, M. Popovic, and R. C. Gallo.** 1983. Persistent in-vitro infection by human T-cell leukaemia-lymphoma virus (HTLV) of normal human T-lymphocytes from blood relatives of patients with HTLV-associated mature T-cell neoplasms. Int. J. Cancer 31:171–180.
31. **Saida, T., K. Saida, M. Funauchi, E. Nishiguchi, M. Nakajima, S. Matsuda, M. Ohta, K. Ohta, H. Nishitani, and M. Hatanaka.** 1988. HTLV-1 myelitis: isolation of virus, genomic analysis and infection in neural cell cultures. Ann. N.Y. Acad. Sci. 540:636–638.
32. **Sanders, M. E., M. W. Makgoba, and S. Shaw.** 1988. Human naive and memory T cells: reinterpretation of helper-inducer and suppressor-inducer subsets. Immunol. Today 9:195–199.
33. **Simmonds, P., P. Balfe, J. F. Peutherer, C. A. Ludlam, J. O. Bishop, and A. J. Leigh Brown.** 1990. Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers. J. Virol. 64:864–872.
34. **Smith, S. H., M. H. Brown, D. Rowe, R. E. Callard, and**

P. C. L. Beverley. 1986. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. Immunology **58**:63–70.

35. **Sugamura, K., M. Fujh, N. Kobayashi, M. Sakitani, M. Hatanaka, and Y. Hinuma.** 1984. Retrovirus-induced expression of interleukin 2 receptors human B-cell lineage. Proc. Natl. Acad. Sci. USA **81**:7441–7445.
36. **Sugamura, K., M. Sakitani, and Y. Hinuma.** 1984. Microplate method for retrovirus-induced transformation of normal human T cells. J. Immunol. Methods **73**:379–385.
37. **Varmus, H., and R. Swanstrom.** 1982. Replication of retroviruses, p. 369–512. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Yamamoto, N., T. Matsumoto, Y. Koyanagi, Y. Tanaka, and Y. Hinuma.** 1982. Unique cell lines harbouring both Epstein-Barr virus and adult T-cell leukaemia virus, established from leukaemia patients. Nature (London) **299**:367–369.
39. **Yoshida, M., M. Seiki, K. Yamaguchi, and K. Tokatsuki.** 1984. Monoclonal integration of human T cell leukemia provirus in all primary tumours of adult T cell leukaemia suggests causative role of human T cell leukemia virus in disease. Proc. Natl. Acad. Sci. USA **81**:2534–2537.
40. **Zack, J. A., S. J. Arrigo, S. R. Weitsman, A. S. Go, A. Haislip, and I. S. Y. Chen.** 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell **61**:213–222.