CONFIGURATION AND EXPRESSION OF THE T CELL RECEPTOR β CHAIN GENE IN HUMAN T-LYMPHOTROPHIC VIRUS I-INFECTED CELLS

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The human T cell leukemia/lymphotropic virus type I (HTLV-I)[1] is a retrovirus etiologically associated with adult T cell leukemia/lymphoma (ATLL) (1). ATLL has an aggressive clinical course and is often accompanied by opportunistic infections (2, 3). Leukemic cells from patients are usually T3,T4$^+$ and T8$^-$ (4); they express IL-2-R and class II HLA (5). This surface phenotype corresponds to that of mature helper/inducer cells that are immunologically activated (6–8). Cell lines derived from the leukemic cells, and cells infected by HTLV-I in vitro, generally have a similar phenotype (9, 10).

Various abnormalities of immune function are demonstrable in the neoplastic cells from ATLL patients and in cell lines infected by HTLV-I. Although the leukemic cells have the surface phenotype of helper cells, they do not manifest helper activity, and some function as suppressor cells (5). Cloned T cells of known antigen specificity that have been infected by HTLV-I in vitro show loss of the ability to respond to specific antigen; both helper and cytotoxic functions may be inhibited (11–14). Some T cells infected by HTLV-I in vitro have been shown to respond to antigen without the requirement for accessory cells (12). However, with time in culture, they also became nonresponsive to antigen. Despite the impairment of antigen-specific responsiveness, some cell lines infected by HTLV-I possess the ability to react nondiscriminatively to HLA class II antigens (15).

Patients hospitalized with specific infectious diseases in an area of Japan in which HTLV-I is endemic were found to have an almost threefold higher incidence of antibodies to HTLV-I than the general population (16). This suggests that immune dysfunction may also be an important consequence of HTLV-I infection in the clinical situation.

T lymphocytes, unlike B lymphocytes, recognize foreign antigen in association with self HLA molecules (17). The T cell receptor (TCR), which is thought to recognize both antigen and HLA determinants (18–20), has recently been identified as a 90 kD surface glycoprotein (21–25). It is composed of two

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[1] Abbreviations used in this paper: ATLL, Adult T cell leukemia/lymphoma; HTLV, Human T cell leukemia/lymphoma (lymphotropic) virus; PBMC, peripheral blood mononuclear cell; TCR, T cell receptor.

disulfide-linked peptides, an α chain of 49–53 kD, and β chain of 43 kD. These are distinct from, but related to, Ig H and L chains (26, 27). The TCR is present on the surface of T cells, and is noncovalently associated with the T3 complex of glycoproteins (21–24, 28).

Extensive molecular characterization of the gene encoding the β chain of the TCR (TCRβ), in both mouse and man, has revealed that it is composed of separate V, D, J, and C region gene segments (29–39). In germline DNA, there is an unknown number of V and D regions; there are two tandemly arranged C region gene segments (Cβ1 and Cβ2), each preceeded by a cluster of J segments. During T cell ontogeny, single V, D, and J segments undergo somatic rearrangement to form a functional gene. This is analogous to the rearrangement of Ig genes in B cells (40). Rearrangement is evident at stage II of intrathymic development, when cells are T3⁻, T4⁺, T6⁺, and T8⁺, but not at an earlier stage of ontogeny (41). The TCRβ gene encodes a 1.3 kb mRNA transcript, which is present at relatively high levels during intrathymic development and at much lower levels in mature, peripheral blood T cells (29, 42). A truncated 1.0 kb mRNA found mainly in immature thymocytes and lacking a V region, is also sometimes transcribed (43). The significance of this transcript is unclear, and a translation product has not been identified.

We investigated the impairment of immune function in HTLV-I-infected cells and the possible role of the TCR in transformation by HTLV-I by addressing several questions. (a) Is there a restricted number of TCRβ gene configurations common to all HTLV-I-infected cells? (b) Does HTLV-I infection significantly alter the level of expression of the TCRβ gene at the mRNA level? (c) Is the pattern of rearrangement or level of expression of the TCRβ gene altered following infection by HTLV-I in vitro? (d) Is the TCRβ gene configuration the same in primary leukemic cells as in the cell lines that are subsequently established in culture?

Materials and Methods

Sources of Cells. HUT 102 (44), MJ (9), UK 69 (45), W4489, WA (9), SD-line (46), and SK-line (9) are all HTLV-I-producing human T cell lines derived from the leukemic cells of patients with ATLL. C91/PL is an HTLV-I-infected cell line established by cocultivation of umbilical cord blood cells with x-irradiated infected cells (9). HUT 78 is an HTLV-I⁻ human neoplastic T cell line that has a similar surface phenotype to the HTLV-I⁺ T cell lines (47). CCRF-CEM and RPMI 8402 are HTLV-I⁻ human T cell lines that have phenotypic markers typical of less mature T cells (48). CR-B (49), CF-B, and RPMI 8392 (48) are EBV-transformed B cell lines that are not infected by HTLV-I. CF-2 and HLS-line are cell lines, established from the peripheral blood of two patients with ATLL, which express B cell antigens and IgL chains (κ on CF-2 and λ on HLS-line) (50). Both cell lines contain integrated HTLV-I proviral sequences and express EBV nuclear antigen. CR-B and CF-B are from the same donors as the HUT 102 and CF-2 cell lines, respectively.

F9164, CF-fresh, HLS-fresh, SD-fresh, and SK-fresh are primary tumor cells from ATLL patients, which were obtained as described previously (51). Fresh normal human lymphocytes were obtained from peripheral blood using Lymphocyte Separation Medium (Litton Bionetics Inc., Kensington, MD) and were cultured for 5 d in complete medium (RPMI 1640 supplemented with 4 mM L-glutamine, 5×10^{-5} M 2ME, 50 U/ml penicillin, and 50 μg/ml streptomycin) with 10% FCS and a 1:1,000 dilution of PHA in the presence of IL-2 (lectin-depleted; Cellular Products, Inc., Buffalo, NY).

Generation of Antigen-specific Helper-inducer Clones. YTA1, TM3, and TM5 are immunologically competent cloned T cells of helper-inducer phenotype, which are specific for tetanus toxoid. They were infected in vitro by cocultivation with lethally irradiated HTLV-I-producing tumor cells, and subsequently designated YTA1-H, TM3-H, and TM5-H, respectively. YTA1 and YTA1-H have been described elsewhere (12). Clones TM3 and TM5 were generated in a similar manner. Briefly, 10^6 peripheral blood mononuclear cells (PBMC) isolated from heparinized blood of a normal volunteer (who had been immunized with tetanus toxoid and whose serum was negative for antibodies to HTLV-I) were cultured with 2 limit flocculation units (Lf) per milliliter of tetanus toxoid (Commonwealth of Massachusetts Department of Public Health, Jamaica Plain, MA) in 24-well microculture plates (Costar, Cambridge, MA) at 37°C in 5% CO_2-containing humidified air in 1 ml of complete media containing 10% autologous plasma. After 7 d in culture, the cells were restimulated with the same concentration of tetanus toxoid plus irradiated (4,000 rad) fresh autologous PBMC. On day 14 and beyond, the cells were exposed to 15% IL-2, and 15% FCS was substituted for the autologous plasma. The cells were restimulated every 7 d as described above. On day 40 in culture, the cells were cloned by limiting dilution. The most rapidly growing clones (TM3, TM5), which were from wells plated with 0.5 cells/well, were expanded with 15% IL-2-containing media and subjected to this study.

Nucleic Acid Analysis. High molecular weight DNA was extracted as previously described (52). For Southern blots, 10–20 μg of DNA were digested for 15 h at 37°C with 2 U/μg of the restriction endonuclease Bam HI, Hind III, or Eco RI (International Biotechnologies, Inc., New Haven, CT); where possible, all three enzymes were used. The digestions were subjected to electrophoresis overnight at 35 V in 0.8% agarose, and transferred to nitrocellulose. They were subsequently hybridized for 15 h at 37°C in 50% formamide/0.45 M NaCl with a ^{32}P-nick-translated probe. After hybridization, filters were washed extensively with 0.5× SSC, 0.1% SDS at 65°C (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7). Hybrid bands were detected by autoradiography with Kodak AR film in a cassette using a Dupont Quanta III screen.

Total cellular RNA was extracted by the guanidine isothiocyanate procedure and further purified by oligodeoxythymidylic acid cellulose chromatography (53). Northern blot analyses were performed as described previously (54). Dot blots were performed as previously described using total cellular RNA, which was quantitated by hybridization with the excised insert from the HLA class I cDNA clone, pDPO (51). Northern blots and dot blots were washed first with 2× SSC, 0.1% SDS at room temperature, then with 0.1× SSC, 0.1% SDS at 42°C, dried, and autoradiographed as for the Southern blots.

The plasmid YTJ2 was kindly provided by Dr. Tak Mak, Ontario Cancer Institute, Toronto, Canada. This is a cDNA clone, in pBR322, of the TCRβ gene isolated from the T cell line Jurkat. It corresponds to nucleotides 100–870 of the published sequence of clone YT35 and includes V and C region sequences (29). The cloned insert was excised with Pst I and purified, using DEAE membrane NA45 (Schleicher and Schuell, Keene, NH), for use as probe.

Analysis of Surface Antigens by mAb. Phenotypic analysis was performed by using indirect immunofluorescence with a FACS (FACS II; Becton-Dickinson Immunocytometry Systems, Sunnyvale, CA), as previously described (55). Briefly, cells were incubated with mAb (50–200 ng of antibody to 2 × 10^5 cells), then washed and treated with an affinity-purified fluorescein-conjugated IgG fraction of goat anti–mouse IgG (whole molecular; Sigma Chemical Co., St. Louis, MO). Control staining with nonreactive mouse ascites at the equivalent protein concentration was consistently negative. The cells were tested with the following antibodies: OKT3, OKT4, OKT8 (Ortho Pharmaceuticals, Raritan, NJ), HLA-DR (Becton-Dickinson Immunocytometry Systems), and anti-Tac, an mAb that reacts with an epitope on or closely linked to the IL-2-R (7). The relative expression of certain surface antigens was compared by median fluorescence intensity (channel number). The value obtained for infected cells was divided by that of uninfected cells; the resultant ratio defines the relative difference, in T3 or T4 antigen expression, between the infected and uninfected cells.
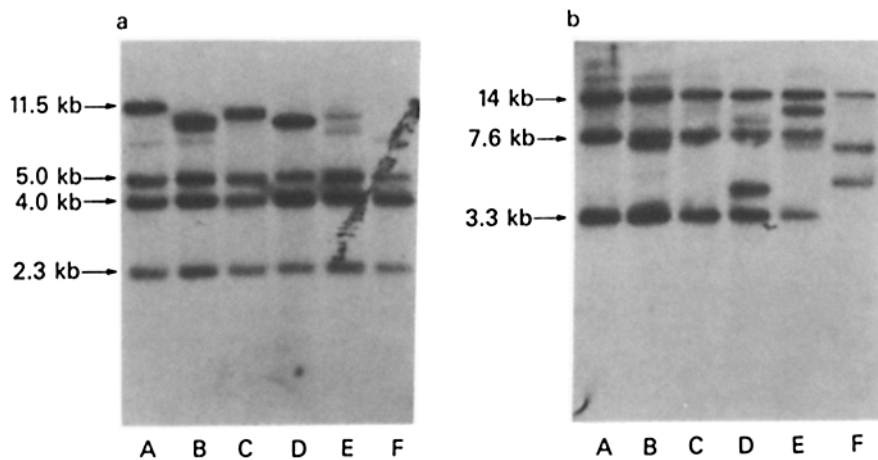
FIGURE 1. Configuration of the TCRβ gene in HTLV-I-infected cell lines. 20 μg of the indicated DNA were digested with Eco RI (a) or Hind III (b) and analyzed by Southern blotting using the TCRβ gene clone YTJ2 insert as probe. The DNA was from CR-B, showing the germline configuration of the TCRβ gene (lane A), HUT 102 (B), MJ (C), UK 69 (D), W4489 (E), and WA (F).

Results

The configuration of the TCRβ gene was studied by Southern blot analysis in 31 cell lines and fresh leukemic cells infected by HTLV-I. Infection by HTLV-I was confirmed in all cases by Southern or Northern blot analysis using a full-length proviral clone as probe. Fig. 1, which shows the arrangement of the TCRβ gene in five representative HTLV-I-infected cell lines, illustrates the main features seen in all Southern blots. Hybridization of DNA from the B cell line CR-B after digestion with Eco RI, and labelling with the TCRβ probe reveals four bands. The band at 11.5 kb contains the Cβ1 gene segment, whereas the band at 4 kb spans the Cβ2 gene segment (39). Bands at 5 kb and 2.3 kb represent hybridization with V gene segments. This pattern of bands is characteristic of the germline arrangement of the TCRβ gene. In the five HTLV-I-infected cell lines shown here, the 11.5 kb fragment is lost after digestion with Eco RI. This indicates that both copies of the Cβ1 segment have either been deleted or rearranged in these cell lines (assuming no chromosomal loss). In Fig. 1, lane B, with DNA from HUT 102, a doublet, consisting of fragments of 9.4 and 8.6 kb is seen in addition to the remaining germline bands; similarly in lane C, with DNA from MJ, two additional bands, again comprising a doublet, are seen. This is consistent with rearrangement of both alleles of the TCRβ gene, and suggests that these rearrangements have used the Cβ1 gene segment. In Fig. 1, lane D, showing DNA from UK 69, a single rearranged band is seen, and there is complete absence of the 11.5 kb germline fragment. In lanes E and F, with DNA from W4489 and WA, respectively, the predominant bands remaining are those representing hybridization with V region gene segments and the fragment spanning the Cβ2 gene segment. This implies that rearrangement of the TCRβ gene in these cell lines has involved complete deletion of the Cβ1 gene segment and use of the Cβ2 gene segment. In Fig. 1, lane E, two faint bands are seen at 10 and 8 kb, indicating that not all of the cells within the cell line have the same
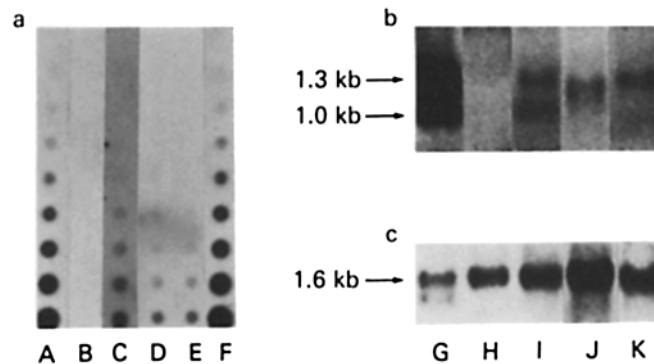
FIGURE 2. Expression of TCRβ gene RNA in HTLV-I-infected cell lines. (a) Twofold serial dilutions of the RNA solutions were subjected to dot blot analysis using the ^{32}P-nick-translated probe YTJ2 insert. RNA was quantitated relative to hybridization with the HLA class I gene pDPO. The RNA was from HUT 78 (lane A), CR-B as a negative control (B), PHA-stimulated lymphocytes (C), HUT 102 (D), MJ (E), and CEM (F). (b) 10 μg of the indicated poly(A)[+]-selected RNA were subjected to Northern blot analysis using YTJ2 insert as probe. (c) Hybridization of the probe pDPO insert to the 1.6 kb HLA class I mRNA was used to estimate the amount of RNA on the filter shown in b. RNA was from RPM1 8402 as a positive control (lane G), RPMI 8392 as a negative control (H), HUT 102 (I), MJ (J), and C91/PL (K). The faint, lower band in c, lane G represents residual hybridization to the TCRβ mRNA.

rearrangement, and suggesting that this is not a clonal population of cells. One cannot exclude the possibility, however, that the cell line was derived from a clonal population of T cells in which further rearrangements of the TCRβ gene occurred with time in culture.

Fig. 1 b shows DNA from the same cell lines digested with the restriction enzyme Hind III. In the germline configuration, the fragment at ~7.6 kb represents hybridization with the 5' part of the Cβ2 gene segment (39). The pattern of bands seen in lane C of Fig. 1 b, showing DNA from MJ, is the same as that of the germline configuration, indicating that TCRβ gene rearrangement is not detectable in DNA from this cell line when cleaved by Hind III. In all the other cell lines shown, additional fragments are seen, implying rearrangement of the TCRβ gene. In Fig. 1 b, lane F, there is complete absence of the 7.6 kb fragment, supporting the interpretation that rearrangement of both alleles, in this example, involves the Cβ2 gene segment. In all the cell lines illustrated, the TCRβ gene is rearranged, and in each case the rearrangement is distinct; furthermore, the Cβ1 and Cβ2 segments appear to be used at roughly equal frequencies.

To determine whether the level of transcription or the nature of the TCRβ gene message is altered in HTLV-I-infected cells, we compared cell lines infected by HTLV-I with non–HTLV-I-infected leukemic cell lines and with PHA-stimulated lymphocytes. Levels of expression of the TCRβ mRNA were compared by dot blot analysis (Fig. 2a). The HTLV-I-infected cell lines HUT102 and MJ express levels of TCRβ RNA comparable to or slightly lower than PHA-stimulated lymphocytes and substantially lower than noninfected cell lines, with phenotypic markers characteristic of similar (Fig. 2a, lane A) and more immature (lane F) T cells. A Northern blot of three HTLV-I-infected cell lines hybridized with the TCRβ gene probe is shown in Fig. 2b. In lane G, showing RNA from
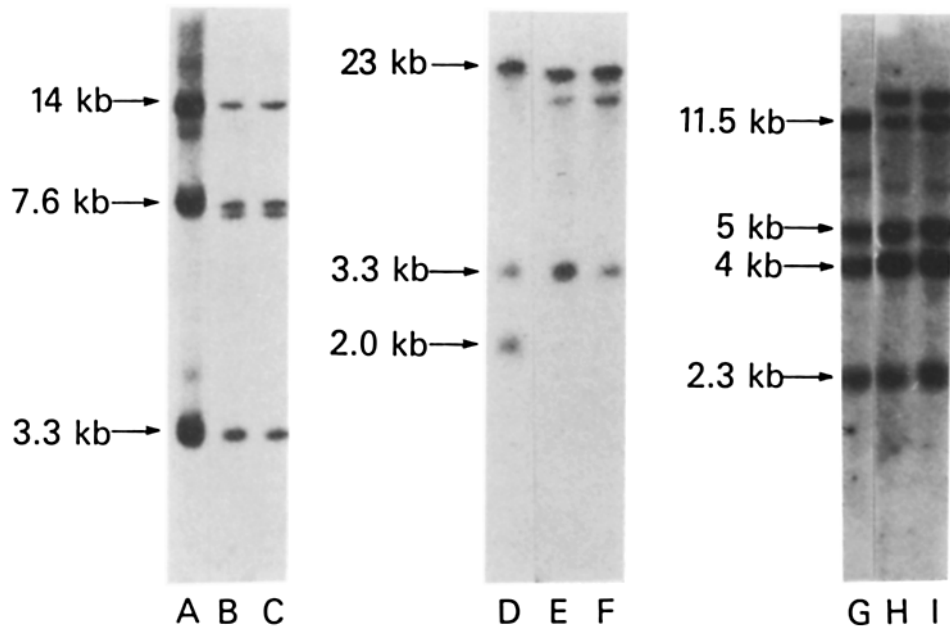
FIGURE 3.   Comparison of the configuration of the TCRβ gene in cloned T cells before and after infection by HTLV-I. 10 μg of the indicated DNA were digested with Hind III (lanes *A–C*), Bam HI (*D–F*), or Eco RI (*G–I*) and subjected to Southern blot analysis using the TCRβ gene clone YTJ2 as probe. The DNA was from CR-B, showing the germline configuration of the TCRβ gene (*A*, *D*, and *G*), clone YTA1 (*B*), YTA1-H (*C*), clone TM3 (*E*), TM3-H (*F*), clone TM5 (*H*), and TM5-H (*I*).

the noninfected T cell line RPMI 8402, an intense band is seen at 1.3 kb, and a faint band is detectable at 1.0 kb. In contrast, in Fig. 2*b*, lanes *I–K*, showing RNA from HTLV-I-infected cell lines, the band at 1.3 kb is less intense. This supports the dot blot analysis and is consistent with these cells being mature T cells. In lane *I*, showing RNA from HUT 102, a band of similar intensity to the 1.3 kb band is seen at 1.0 kb. This most probably represents a transcript lacking a V region, and is an unexpected finding in a mature T cell.

The patterns of rearrangement and expression of the TCRβ gene were compared in well-characterized T cell clones before and after in vitro infection by HTLV-I to correlate any changes with alterations in immune function. YTA1, TM3, and TM5 are T cell clones of helper-inducer phenotype that are specific for tetanus toxoid. They display the following surface phenotype: OKT4$^+$, OKT3$^+$, OKT8$^-$, HLA-DR$^+$, and Tac-antigen$^+$. Following in vitro infection by HTLV-I, clone YTA1 (now called YTAI-H) lost the requirement of accessory cells for proliferation in response to tetanus toxoid (12). After a longer time in culture, this T cell clone proliferated spontaneously and lost all antigen-specific responsiveness. Clones TM3-H and TM5-H were unable to respond to specific antigen a short time after infection by HTLV-I (data not shown). In addition, TM3-H and TM5-H appeared to react nondiscriminatively to HLA class II antigens (our unpublished observations). Fig. 3 shows a comparison of the TCRβ gene rearrangements before and after infection by HTLV-I. In DNA from each clone an additional band is seen relative to the germline configuration, when the
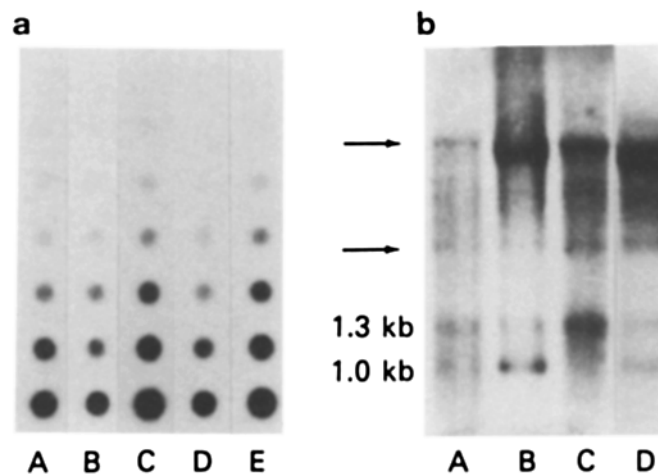
FIGURE 4. Comparison of expression of the TCRβ gene mRNA in cloned T cells before and after infection by HTLV-I. (a) Dot blot analysis. RNA solutions were spotted in vertical rows using twofold serial dilutions. The filter was hybridized with the ^{32}P-nick-translated probe YTJ2 insert, which contains both C and V regions of the TCRβ gene. RNA was quantitated relative to hybridization with the HLA class I gene pDPO. The RNA was from clone TM3 (lane A), clone TM3-H (B), clone TM5 (C), clone TM5-H (D), and PHA-stimulated lymphocytes as a control (E). (b) Northern blot analysis. ~10 μg of total cellular RNA from the indicated T cell clones were subjected to Northern blot analysis using YTJ2 insert as probe. Arrows indicate the positions of the 28 S and 18 S markers. The RNA was from TM3 (lane A), TM3-H (B), TM5 (C), and TM5-H (D).

appropriate restriction enzyme is used; this implies rearrangement of the TCRβ gene. No difference in the pattern of bands is detectable after infection by HTLV-I. A comparison of the levels of expression of TCRβ RNA in clones TM3 and TM5 pre- and postinfection is shown in Fig. 4a. After infection, expression appears reduced by approximately twofold relative to the expression of HLA class I genes; it is unlikely that this represents a significant decrease. Fig. 4b shows a Northern blot analysis of RNA from the same T cell clones. In the uninfected clone TM3, transcripts of 1.3 and 1.0 kb are present. After infection, both mRNA species are transcribed, but the 1.0 kb transcript appears relatively more abundant. In the uninfected clone TM5, only the full-length mRNA is detectable; however, after infection by HTLV-I, both transcripts are visualized at roughly equal intensity.

Surface expression of the T3 complex in these T cell clones was studied by FACS analysis, as this complex of glycoproteins is stoichiometrically expressed with and noncovalently linked to the TCR on the surface of immunocompetent T cells (21–24, 28). Expression of T3 molecules by infected cells was at least 74% of that of the uninfected cells of the same lineage when compared by median fluorescence intensity (Fig. 5). A small percentage of cells within each of the infected cell populations had a reduced density of surface T3 molecules, which resulted in a slight splaying of the fluorescence profile relative to the homogeneous T3 expression observed in the uninfected counterparts. Infected cells also retained their expression of the T4 surface determinant.

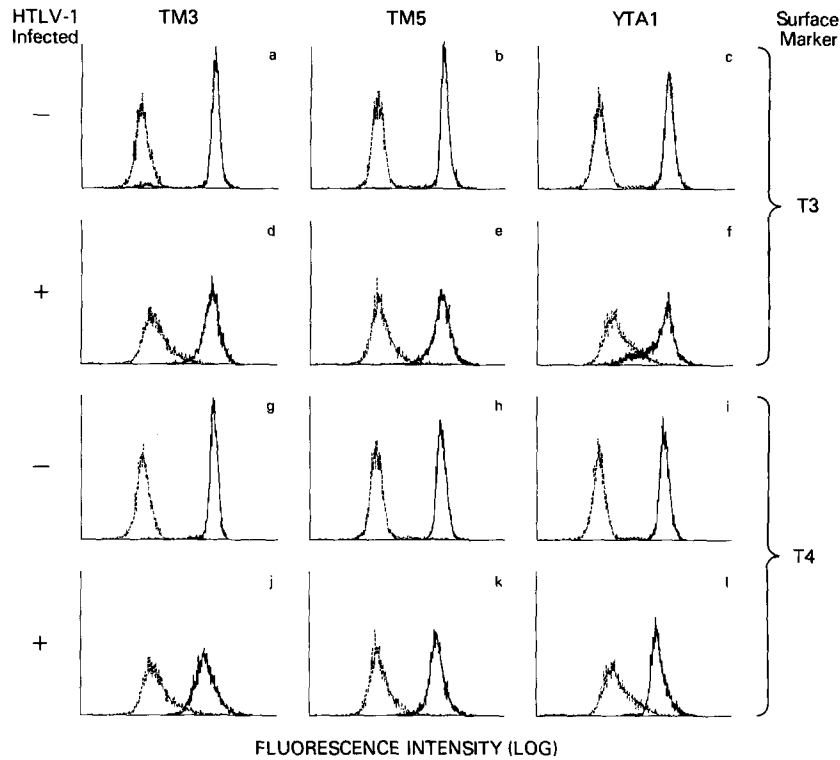The configuration of the TCRβ gene in fresh tumor cells from ATLL patients

FIGURE 5. The effect of HTLV-I infection on the expression of cell surface T3 and T4 determinants was analyzed by indirect immunofluorescence and flow cytometry. Cell lines TM3, TM5, and YTA1 were either uninfected (−) (a–c, g–i) or infected (+) (d–f, j–l) with HTLV-I and stained for reactivity with antibodies OKT3 (a–f) or OKT4 (g–l) (solid lines). As a negative control, unreactive mouse ascites was substituted for primary antibody (broken lines). Fluorescence intensity is plotted on a logarithmic scale (abscissa), and the ordinate represents cell number.

was compared with the configuration in the cell lines subsequently established in culture. The first two examples, CF and HLS, have been the subjects of previous reports (50, 56). The cell lines established from these cases are unusual in that they have surface markers characteristic of B cells. In both, DNA from the fresh tumor cells (Fig. 6) shows a clonal pattern of rearrangement of the TCR gene, indicated by the appearance of additional fragments and loss of germline fragments. However, the configuration of the TCRβ gene in the derived cell lines is identical to that of the germline configuration, consistent with these cells being of B cell origin as previously documented (50, 56). In Fig. 6, lanes g–l, showing a Southern blot analysis of DNA from patient SK, both the fresh tumor cells and the autologous cell line have clonal patterns of rearrangement of the TCRβ gene. The single rearranged fragment in DNA from the primary tumor cells is of a different size from the two additional fragments seen in DNA from the derived cell line, indicating that the TCRβ rearrangements that have occurred in these two cell populations are distinct. This strongly suggests that the cell line did not arise from the primary tumor cells. Again, from this data one cannot exclude the possibility that further rearrangements occurred in culture, but a
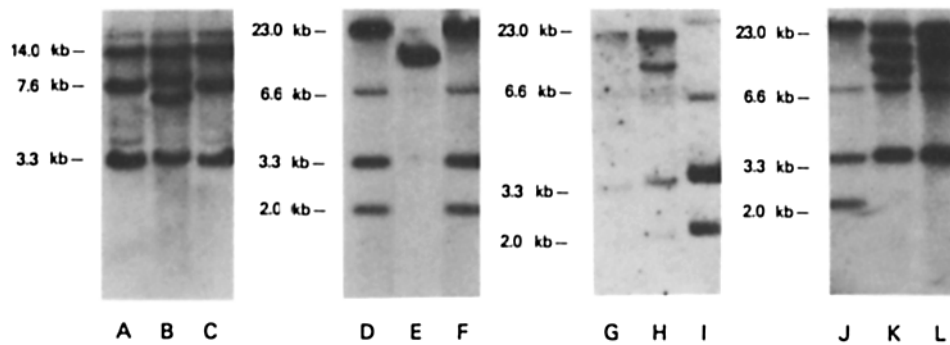
FIGURE 6. Comparison of the rearrangement of the TCRβ gene in primary tumor cells and derived cell lines. 20 μg of the indicated DNA were digested with Hind III (lanes A–C) or Bam HI (D–L) and analyzed by Southern blotting using the TCRβ gene clone YTJ2 insert as probe. The DNA was from CF-B, showing the germline configuration of the TCRβ gene, (A), CF-fresh (B), CF-2 (C), CR-B again showing the germline configuration (D, G, and J), HLS-fresh (E), HLS-line (F), SK-fresh (H), SK-line (I), SD-fresh (K), and SD-line (L).

comparison of proviral integration sites in these cells is more consistent with the former interpretation (57).

Patient SD (Fig. 6d) was of particular interest because karotypic analysis of the fresh tumor cells revealed a tandem duplication of the long arm of chromosome 7 (58, 59). The TCRβ gene has been mapped to chromosome 7 (60); conflicting reports have placed the gene variously on the long and the short arm (61, 62). Southern blot analysis following Bam HI digestion of DNA from primary tumor cells reveals the presence of two rearranged bands, in addition to the germline bands. This suggests that at least three differently arranged alleles of the TCRβ gene are present in the tumor cells. The cell line established from this patient has a normal karyotype and a TCRβ gene rearrangement, with evidence of only two alleles, which is different from that seen in the primary tumor cells. This again implies that the cell line was not derived from the primary tumor cells.

The configuration of the TCRβ gene in DNA from fresh neoplastic cells from four patients with ATLL is shown in the above comparison (Fig. 6, lanes B, E, H, and L), and one additional case, F9164, was also studied (data not shown). In each case, rearrangement of the TCRβ gene is indicated by the loss of germline fragments and the presence of additional rearranged fragments. The tumor cells appear clonal with respect to TCRβ gene rearrangement in all digestions, and no common pattern of rearrangement is observed.

Discussion

The mechanism of transformation by HTLV-I is unknown. The viral genome does not contain an oncogene of cellular origin and there is no evidence that promoter-enhancer insertion is operative in this disease, as integration of the virus does not seem to occur at a specific site in the host cell DNA. It has been postulated that the product of the x-lor gene at the 3' end of the viral genome acts by trans-acting transcriptional activation of viral and cellular genes, in particular genes that are specific for T cell maturation and proliferation (63). In this study, we found that the full length TCRβ mRNA was transcribed at similar

or slightly lower levels by HTLV-I-infected cell lines than by PHA-stimulated lymphocytes; this suggests that this T cell–specific gene is not transcriptionally activated or significantly depressed, as a result of HTLV-I infection.

Infection by HTLV-I in vitro results in a variety of abnormalities of immune function, including impairment of the response to specific antigen and acquisition of alloreactivity. Levels of TCRβ gene mRNA and surface T3 expression do not appear significantly altered after HTLV-I infection. Taken together, these data suggest that the number of TCR molecules on the cell surface is not appreciably changed after HTLV-I infection. Weiss and Stobo (64) have recently described a mutagenized Jurkat clone that expressed approximately one-tenth the density of TCR and T3 determinants expressed by the wild type Jurkat. Despite this, it could be activated to levels comparable to those observed in the wild type Jurkat cells by both anti-T3 and clonotypic antibodies, and also by PHA plus PMA. It is unlikely, therefore, that a subtle change in the number of TCR on the cell surface could account for loss of antigen-specific responsiveness. Thus, alterations in immune function in HTLV-I-infected cells must be due either to structural changes in the TCR-T3 complex or to altered expression of other molecules involved in the response to antigen. Comparison of the TCRβ gene configurations before and after HTLV-I infection does not reveal any changes detectable by Southern blot analysis. We cannot exclude the possibility that somatic mutations have occurred within the β chain gene or that changes have occurred within the α chain gene. It has been previously reported (65) that loss and gain of alloreactivity, among clones derived from a T cell hybridoma, were associated with structural changes in both α and β chains of the TCR, even though Southern blot analysis of the β chain gene did not detect any differences. It is possible that similar sorts of somatic mutation, resulting in structural changes in the TCR which are not detectable at the level of Southern blotting, occur after HTLV-I infection.

The appearance of the 1.0 kb TCRβ transcript, in addition to the full length mRNA in clone TM5-H is an intriguing finding. There is no evidence that this mRNA is translated, and it may simply represent a transcript from a nonfunctional rearrangement of the TCRβ gene. However, after infection, this clone appears to be nondiscriminatively alloreactive, as does the cell line HUT102, which also expresses the truncated message. One could speculate that the encoded peptide chain forms part of a receptor complex capable of binding to a common structural determinant on HLA class II genes. Also, this transcript may represent a gene that is transcriptionally activated by the viral x-lor gene product.

We have studied the configuration of the gene encoding the TCRβ gene in the primary tumor cells from five cases of ATLL, and in 26 cell lines infected by HTLV-I, 12 of which are shown in this report. In all of these, with the exception of two cell lines that have B cell markers, the TCRβ gene was rearranged. In most of the cell lines and in all of the fresh tumor cells examined, the cell populations appeared clonal with respect to a particular TCRβ gene rearrangement. All of the primary tumor cells and cell lines established from ATLL cases differed in the configuration of the TCRβ gene. This suggests that at least the final clonal expansion of T cells associated with transformation by HTLV-I occurs after TCRβ gene rearrangement.

Studies on TCR gene rearrangements provide a unique way of identifying individual T cells. We used this property to compare primary leukemic cells from ATLL patients with the cell lines that were subsequently established from them. Our finding that, in all of the instances examined, the TCRβ gene rearrangements in the primary cells were different from those in the cell lines suggests that the cells that grow out in culture are often, perhaps usually, not the original neoplastic cell. This extends previous studies (58, 60) that compared proviral integration sites and karyotypic abnormalities between primary cultures and derived cell lines. It is possible that the transformed cells that grow out in culture become infected by HTLV-I in vitro, and that recent infection by HTLV-I confers a growth advantage over the cells previously infected in vivo.

SD is particularly interesting in that the primary tumor cells had a tandem duplication of the long arm of chromosome 7 and evidence of three differently arranged alleles of the TCRβ gene on Southern blot analysis. The latter result can be interpreted in several ways. (*a*) The fresh tumor cells may be a biclonal population of cells, with two distinct TCRβ gene rearrangements. (*b*) A second TCRβ gene rearrangement may have occurred during proliferation of the tumor cells in vivo. (*c*) A chromosomal break may have occurred within the TCRβ gene. (*d*) The tandem duplication of the long arm of chromosome 7 may have resulted in the presence of three alleles of the TCRβ gene. The last interpretation seems most likely in view of the karyotypic abnormality, the intensity of the bands seen on Southern blot analysis, and the presence in these cells of a single integrated provirus (data not shown). If this is indeed the case, the presence of three differently arranged TCRβ genes suggests that the tandem duplication preceeded TCRβ gene rearrangement. This chromosomal abnormality was not present in all of the patient's T cells, since the T cell line established in culture had a normal karyotype (59). Chromosomal abnormalities are demonstrable in nearly every case of ATLL; chromosome 7 is one of the chromosomes most frequently involved, but no consistent alterations have emerged (58, 59, 66). Nowell et al. (59) postulated that a chromosomal change is not a required early step in the pathogenesis of ATLL, but may be involved in later stages of tumor progression. If this is true in the case of SD, then it would imply that not only the chromosomal change but also the initiation of the malignant process, which is likely to be a result of infection by HTLV-I, occurred before TCRβ gene rearrangement, at an early stage of T cell maturation. Since fresh and cultured cells from patients with ATLL, and umbilical cord blood cells infected by HTLV-I are T4$^+$, it has been suggested that the in vivo transformation process occurs in mature peripheral blood cells (67). However, Markham et al. (67) showed that bone marrow cells lacking the T4 and T8 phenotype, i.e., more immature cells, can be targets for transformation by HTLV-I. It is possible, and in keeping with the above data, that the infective process involves an immature cell, which subsequently rearranges its TCR genes, matures, and expresses the more commonly observed phenotype.

The receptor-mediated theory of leukemogenesis proposes that the envelope of slowly transforming retroviruses interacts with immunospecific receptors and/ or growth factor receptors on the surface of B and T cell neoplasms to cause continued cell division (68). If this were applicable to HTLV-I-associated malig-

nancies and the TCR molecule, one might expect to find a limited number of TCR gene arrangements in cells infected by HTLV-I. All of the fresh tumor cells and cell lines established from the ATLL cases we examined had distinct TCRβ gene configurations. This result implies that infected cells can have a very large number of different TCRβ gene rearrangements, and makes the above hypothesis unlikely in the case of ATLL. However, since the TCR most probably has the capacity to recognize both foreign antigen and self HLA determinants, differences in gene rearrangement may reflect differences in HLA restriction and not simply different antigenic specificities. Therefore, we cannot exclude the possibility that all of the TCR gene rearrangements described here might result in a molecule capable of binding to determinants on the viral envelope, although the extent of these differences makes this seem unlikely.

Several aspects of the role of the TCR in alterations of immune function after HTLV-I infection and in transformation by HTLV-I remain speculative. However, several facts have emerged. There is not a restricted number of patterns of rearrangement of the TCRβ gene associated with transformation by HTLV-I. Expression of the full length TCRβ mRNA is not significantly altered in cells transformed by HTLV-I. Cell lines derived from the leukemic blood cells of ATLL patients are frequently, and perhaps always, different from the actual leukemic cells. We hope that future studies will shed light on the molecular events that lead to changes in immune function after infection by HTLV-I.

Summary

We studied the configuration and expression of the gene encoding the β chain of the T cell receptor (TCRβ) in cell lines and primary tumor cells infected by the human T cell leukemia/lymphoma (lymphotrophic) virus type I (HTLV-I). Most of the cell lines and all the primary tumor cells showed rearrangement of the TCRβ gene, and in each case the rearrangement was distinct. The majority of cases examined were clonal with respect to a particular TCRβ gene rearrangement. Primary tumor cells from one case (SD) were found to have a tandem duplication of a portion of chromosome 7; this appears to have resulted in the presence of three alleles of the TCRβ gene, each of which is arranged differently. This suggests that the chromosomal abnormality, and possibly infection by HTLV-I, occurred before TCRβ gene rearrangement. Cell lines infected by HTLV-I express levels of TCRβ mRNA similar to PHA stimulated lymphocytes, suggesting that this gene is not transcriptionally activated as a result of infection by HTLV-I.

Cloned T cells of known antigen specificity that are infected by HTLV-I in vitro show impairment of immune function, including loss of antigen-specific responsiveness and the acquisition of alloreactivity. Comparison of the configuration of the TCRβ gene before and after infection revealed no changes detectable by Southern blot analysis. Levels of expression of the TCRβ gene at the mRNA level and surface expression of the T3 complex were also not significantly altered, suggesting that changes in immune function cannot be attributed to quantitative changes in the TCR molecule.

The configuration of the TCRβ gene in primary tumor cells infected by HTLV-I was compared with that in the derived cell lines. In all pairs examined,

the configuration in the primary tumor cells was different from that in the cell lines, strongly suggesting that the cells that grow in culture are not the original neoplastic cells.

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