Phenotypic Progression of a Rat Lymphoid Cell Line Immortalized by Human T-Lymphotropic Virus Type I To Induce Lymphoma/Leukemia-Like Disease in Rats

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Received 1 May 1992/Accepted 7 August 1992

Rat lymphoid cells, TARS-1, immortalized by coculture with adult T-cell leukemia cells, were intraperitoneally injected into 65 newborn, inbred WKAH/Hkm rats. In most of the rats, tumor nodules were discernible 7 to 15 days after transplantation but were completely rejected within 5 to 6 weeks. Two rats with no tumor nodules exhibited gait disturbances and paralysis of the hind legs 3 to 4 weeks after transplantation. Histological and hematological examinations revealed that a lymphoma/leukemia-like disease had developed in one of the two rats, and the T-lymphoid cell line WLeuk-1 was established from peripheral blood mononuclear cells from this rat. When the WLeuk-1 cells were transplanted into newborn WKAH/Hkm rats, the animals died of a lymphoma/leukemia-like disease within several weeks after transplantation, in contrast to their rejection of the TARS-1 cells. Southern blot and karyotype analyses revealed that WLeuk-1 cells had retained the marker chromosomes and human T-lymphotropic virus type I (HTLV-I) integration patterns of the parent cell line, TARS-1. The additional specific chromosome abnormalities 3p+, t(12;13), and Xq+ were found in the WLeuk-1 cells. Moreover, the expression of HTLV-I structural proteins was slightly depressed in WLeuk-1 cells, while that of the transacting factors p40^{tax} and p21^r, but not that of p27^r, was enhanced about fivefold compared with that in TARS-1. The transactivating function of p40^{tax} was intact in WLeuk-1, as evidenced by enhanced interleukin-2 receptor alpha chain expression. These results suggest that aberrant expression of HTLV-I regulatory genes and alteration of cellular genes were associated with the phenotypic progression of the WLeuk-1 cell line.

Human T-lymphotropic virus type I (HTLV-I), a type C retrovirus isolated from adult T-cell leukemia/lymphoma (ATL), can immortalize T lymphocytes and fuse fibroblasts or epithelial cells to produce large syncytia (33). ATL can be classified into four types, according to the clinical symptoms and course of the disease: smoldering, chronic, crisis, and acute, the prototype being the acute form (13). Patients with acute ATL show little response to therapy, and most die shortly after diagnosis, the median survival time being 4.2 months (13). The age specificity of ATL suggests the existence of an age-dependent accumulation of leukemogenic events within HTLV-I-immortalized T lymphocytes; the number of independent leukemogenic events in ATL has been estimated to be five on the basis of analysis by computer simulation (19). The molecular biological basis of the multistep carcinogenesis in ATL remains unclear, despite the advances in research on signal transduction and regulatory mechanisms of HTLV-I gene expression.

HTLV-I is related to other diseases, such as HTLV-I-associated myelopathy/tropical spastic paraparesis (7), HTLV-I-associated bronchopneumonopathy, HTLV-I-associated uveitis, and HTLV-I-associated arthropathy. It has been proposed that these diseases be termed "HTLV-I-related syndrome." The establishment of animal models is an urgent requirement for studies on the prevention and

treatment of these diseases, as well as for fundamental investigations concerning the molecular mechanisms of these diseases and the pathogenesis of HTLV-I. The establishment of a rodent system would facilitate such molecular and immunological investigations. In the study reported here, we induced lymphoma/leukemia-like diseases in rats by injecting HTLV-I-transformed rat lymphoid cells into newborn animals. HTLV-I-bearing T lymphocytes established from the peripheral blood mononuclear cells of rats with experimentally induced lymphoma/leukemia-like diseases showed an aggressive biological phenotype. This is a useful model for investigation of the multistep leukemogenesis of ATL.

MATERIALS AND METHODS

Animals. Inbred WKAH/Hkm rats obtained from SLC Japan Co., Ltd. (Shizuoka, Japan), were maintained at the Institute for Laboratory Animals, Kochi Medical School. On each rat's day of birth, 1×10^7 or 5×10^6 viable rat lymphoid cells were intraperitoneally injected.

Cell line. TARS-1 is a WKA/Hok rat lymphoid cell line established by coculture with 5-bromo-2'-deoxyuridine-treated ATL cells (28). MT-2 is a human umbilical-cord T-cell line established by coculture with fresh ATL cells (16). Jurkat, a gift from J. Hamuro, Ajinomoto Co., Yokohama, Japan, is a human T-cell leukemia cell line. HUT102, kindly provided by N. Yamamoto, Tokyo Medical and

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Dental University, is a human T-cell line established from the peripheral blood of a patient with HTLV-I-associated mycosis fungoides (20). The mononuclear cell fraction of peripheral blood separated with lymphocyte separation medium (Organon Technika Co., Durham, N.C.) was cultured in medium supplemented with 20 U of recombinant human interleukin-2 (IL-2) per ml kindly provided by Takeda Chemical Industries, Ltd. (Osaka, Japan). The lymphoid cell lines used in this study were routinely passaged in RPMI 1640 medium supplemented with heat-inactivated 15% fetal calf serum in a humidified 7.0% CO₂ atmosphere at 37°C.

Titration of antibodies against HTLV-I. A gelatin particle agglutination assay was conducted for the titration of antibodies against HTLV-I, according to the supplier's directions (Fuji Rebio, Inc., Tokyo, Japan).

Immunocytochemistry. Cell surface markers of the cultivated cells were analyzed by the avidin-biotin complex-peroxidase method with Histofine kits (Nichirei Co., Tokyo, Japan) used for the acetone-fixed cell preparations. A variety of mouse anti-rat cell marker monoclonal antibodies were purchased from Serotec, Ltd. (Oxford, United Kingdom): W3/25 (anti-CD4), MRC-OX8 (anti-CD8), MRC-OX6 (anti-major histocompatibility complex [MHC] class II), and W3/13 (anti-rat *panT*). For reference, the human cell surface markers Leu4 (anti-CD3) (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) and OKIa1 (anti-HLA-DR) (Ortho Diagnostic Systems, Raritan, N.J.) were used. Anti-HTLV-I p19 monoclonal antibody (Cellular Products, Inc., Buffalo, N.Y.) was also used.

Southern blot analysis. DNA digested with restriction enzymes was subjected to electrophoresis on a 0.8% agarose gel and then transferred onto Pall-Biodyne nylon membrane filters. Southern hybridization was performed as described previously (18, 25). The 8.2-kb *SacI* fragment isolated from the pMT-2 plasmid (20a) (generously provided by R. C. Gallo, National Institutes of Health, Washington, D.C.), containing almost the full-length genome of HTLV-I, and the constant region of the mouse T-cell receptor (TCR)-beta chain (kindly provided by H. Yamamoto, Kochi Medical School) were ³²P labeled with a multiprimer labeling system (Oligolabelling kit; Pharmacia, Uppsala, Sweden) and denatured before being used.

Western blot (immunoblot) analysis. Western blot analysis was performed according to the technique of Towbin et al.

(29). Following 12.5% polyacrylamide gel electrophoresis of cellular protein lysates from each culture, banded proteins were electrophoretically transferred to polyvinylidene difluoride membrane (Immobilon; Millipore, Ltd., Bedford, Mass.) and then treated with the following antibodies: anti-HTLV-I-positive human serum (1:1,280), Lt-4 (27) (anti-*tax1* mouse monoclonal antibody), MRC-OX6 (anti-rat MHC class II mouse monoclonal antibody) (Serotec), and Rec-6 (anti-HTLV-I *rex1* and p21⁺ rat monoclonal antibody), which was obtained by the standard hybridoma isolation technique from rats immunized with a synthetic oligopeptide of the C-terminal 16 amino acids of *rex*. The immunoreactive bands were visualized with peroxidase-labeled rabbit anti-human immunoglobulin (Ig), goat anti-mouse Ig, or rabbit anti-rat Ig (DAKO PATTS Co., Ltd., Glostrup, Denmark) following reactions with the substrate of the enhanced chemiluminescence Western blotting system (Amersham Co., Ltd., Tokyo, Japan) and subjected to autoradiography. Densitometry was performed with a dual-wavelength Chromato Scanner (CS-930; Shimadzu Co., Ltd., Kyoto, Japan).

Fluorescence-activated cell sorter analysis. Cells were stained at 4°C with the following mouse monoclonal antibodies: MRC-OX18 (anti-rat MHC class I; RT1A), MRC-OX6 (anti-rat MHC class II; Ia), MRC-OX39 (anti-rat IL-2R- α) (Serotec), and fluorescein isothiocyanate-conjugated rabbit anti-mouse Ig. Samples were analyzed with an EPICS-750 (Coulter, Inc., Hialeah, Fla.).

Cytogenetic analysis. Cytogenetic studies were accomplished with rat cell lines. Chromosome spreads of the cell lines were prepared by the standard procedures and stained with a combination of quinacrine mustard and Hoechst 33258 (Boehringer, Mannheim, Germany) (37). Ten to 20 metaphases were karyotyped and analyzed according to the chromosome-numbering system proposed by the Committee for a Standardized Karyotype of *Rattus norvegicus* (5) as well as proposed diagrams (14, 23).

RESULTS

Transplantation of TARS-1 cells. Fifty-two of 65 newborn WKAH/Hkm rats that had been intraperitoneally injected with TARS-1 formed local tumor nodules measuring up to 2.5 cm in diameter each. The nodules were confirmed histologically to be occupied by highly atypical large-cell-

TABLE 1. Characterization of peripheral blood samples from rats injected with HTLV-I-infected cells^a

Rat no.	Cells	No. of inoculated cells	Age at sacrifice (wk)	Anti-HTLV-I Ab titer	Total WBC (10 ³ /μl)	WBC (%)						Remarks
						Band.	Seg.	Lympho.	Mono.	Eosino.	Atypical lympho.	
101	None ^b	0	4	— ^c	7.0	4	12	79	3	2	0	Norm
210	TARS-1	1 × 10 ⁷	3	1:16	25.7	3	69	25	1	0	2	L/L
212	TARS-1	1 × 10 ⁷	5	1:16	15.7	1	10	82	5	2	0	L
215	TARS-1	1 × 10 ⁷	5	1:32	11.2	1	3	95	1	0	0	R
310	TARS-1	1 × 10 ⁷	4	1:16	13.8	0	7	90	2	0	0.5	L
410	TARS-1	5 × 10 ⁶	3	1:16	7.0	0	9	87	3	1	0	R
414	TARS-1	5 × 10 ⁶	52	1:32	ND	ND	ND	ND	ND	ND	ND	R
510	WLeuk-1	5 × 10 ⁶	2	1:16	23.7	0	19	63	14	3	1	L/L
512	WLeuk-1	5 × 10 ⁶	2	1:16	16.7	0	15	77	5	2	1	L
515	WLeuk-1	5 × 10 ⁶	3	1:16	43.7	0	45	39	9	7	0	L
517	WLeuk-1	5 × 10 ⁶	3	1:32	34.9	0	33	53	9	5	0	L

^a Ab, antibody; WBC, leukocytes; band., band neutrophil; seg., segmented neutrophil; lympho., lymphocytes; mono., monocytes; eosino., eosinophils; norm, normal control; L/L, lymphoma/leukemia-like disease; L, lymphoma-like disease; R, rejection after tumor formation; ND, not done. No basophils were found.

^b None, cells were not injected.

^c —, cannot be detected.

FIG. 1. (A and B) Tumor nodules formed in rat 310, which had the gait disturbance. (A) Tumor cells in the nodule, exhibiting strongly atypical large-cell-type lymphoblastic cells; (B) nodular-proliferating focus of tumor cells in the meninges of the cerebellum. (C and D) Large-cell-type lymphoblastic cells in rat 210, which had paralysis of its rear legs. Note the diffuse infiltrative patterns in the hippocampus (C) and the bone marrow (D).

type lymphoblastic tumor cells and were not in an area of swelling and local inflammation. Within 2 to 3 weeks after inoculation, the tumor nodules had begun to decrease in size, and within 5 to 6 weeks, they were no longer palpable. Autopsies performed after 1 year of follow-up examinations verified the complete rejection of the tumors. Eleven of the rats injected with TARS-1 died within several days of inoculation; most died of maternal neglect due to the injection procedure, and others may have incurred injury to organs during the procedure.

Two rats without palpable tumor nodules developed symptoms; rat 210 had paralysis of the rear legs, and rat 310 displayed gait disturbance. These manifestations appeared 3 to 4 weeks after inoculation. The total leukocyte count of rat 210 increased to about three times that of a normal control (rat 101). Neutrophilia was marked, especially among segmented forms, and 2% of the lymphocytes were atypical (Table 1). On the other hand, the total number of leukocytes in rat 310 increased only slightly, and 0.5% were atypical lymphocytes. Anti-HTLV-I antibody titers in serum were positive but low (1:16 or 1:32).

Tumor histology (rats 210 and 310). The histology of tumor specimens obtained from rat 310 (gait disturbance) is shown in Fig. 1A and B. Large, round tumor cells with marked atypia, i.e., lymphoblastic tumor cells, grew compactly in areas containing some necrosis. The tumor cells had infiltrated and formed multiple metastatic nodules in the cerebellar meninges, lungs, liver, and lymph nodes. However, metastatic foci were not detected in the spleen, thymus, bone marrow, stomach, or intestine.

In rat 210 (rear-leg paralysis), the same type of highly atypical lymphoblastic tumor cells had formed compact metastatic nodules in the injection site, peritoneum, liver, lung, kidney, and pancreas. In addition to these lymphoma-

like lesions, diffuse tumor cell infiltration was observed in the bone marrow, lymph nodes, spleen, kidney, and hippocampus (Fig. 1C and D). The tumor cells had diffusely infiltrated around the hippocampus from the adjacent Virchow-Robin's space in a leukemic pattern. The pathologic features in this rat corresponded to those of a leukemia/lymphoma-like disease.

Characterization of WLeuk-1 cells. Our attempt to establish rat lymphoid cell lines from peripheral blood mononuclear cells of rats inoculated with TARS-1 cells succeeded only with rat 210. This cell line, termed WLeuk-1, grew vigorously and became IL-2 independent after 4 weeks in culture. These cells had a large nuclear/cytoplasmic ratio and atypical, lobulated nuclei with prominent nucleoli; they

FIG. 2. Wright-Giemsa staining of lymphoid cells. (A) Atypical lymphocytes with an irregularly lobulated nucleus in the peripheral blood of rat 210; (B) WLeuk-1 cells established from peripheral blood mononuclear cells of rat 210. Bars = 20 μ m.

FIG. 3. Genomic Southern blot analyses. (A) Detection of the HTLV-I proviral genome in TARS-1 and short-term culture of WLeuk-1 preparations. Lane MH, tumor nodule formed in rat 210. MT-2 cells and rat normal peripheral blood lymphocytes (Norm.L.) were used for positive and negative controls, respectively. DNAs from these cells were hybridized with a full-length probe of HTLV-I. (B) Rearrangements of the TCR-beta chain in the HTLV-I-positive rat cell lines. DNAs from normal rat liver, normal rat kidney, TARS-1, and WLeuk-1 cells were digested with several restriction enzymes and fractionated with 0.8% agarose. Southern blots of gels were hybridized with the constant region of the mouse TCR-beta cDNA probe. (C) Proviral DNA of HTLV-I in WLeuk-1 cells at passage 21 after establishment, the same stage at which karyotype and TCR-beta analyses were performed. MT-2 and Jurkat cells were used as positive and negative controls, respectively. Hybridization was performed with a full-length probe of HTLV-I.

resembled the large, atypical lymphoblastic tumor cells observed in the peripheral blood of rat 210 (Fig. 2). The doubling time of the WLeuk-1 cells was 20.9 h, which is somewhat shorter than that of TARS-1 cells (26.5 h).

When 5×10^6 WLeuk-1 cells were injected into 25 newborn WKAH/Hkm rats, all of the animals died of malignant lymphoma-like or lymphoma/leukemia-like diseases within 3 weeks (Table 1). In rats injected with WLeuk-1 cells, neoplastic lymphoblastic cells proliferated and formed compact nodules at the injection sites and in various organs. Histologically, the tumor tissue was identical to that of the tumors from rat 210.

On immunocytochemical evaluation, WLeuk-1 cells were positive for rat *panT*, Ia, and CD4 but negative for rat CD8 and human CD3 and HLA-DR, which is also the case for TARS-1 cells. The results of Southern blot analysis are shown in Fig. 3. When genomic DNA was digested with *EcoRI*, which cleaves at the flanking region of the HTLV-I genome, an additional band not present in the TARS-1 cells

was detected in the short-term primary WLeuk-1 culture at about 3 weeks (Fig. 3A). However, after more than 15 passages, this additional band disappeared and the integration patterns of HTLV-I in WLeuk-1 and TARS-1 cells became identical, as shown by analysis with five different restriction enzymes (Fig. 3C). Analysis of the TCR-beta chain rearrangement (32) revealed that bands corresponding to the constant region of TCR-beta-1 (Cbeta-1) were lost in both TARS-1 and WLeuk-1 cells (Fig. 3B). Thus, both alleles of Cbeta-1 were deleted, and rearrangement of TCR-beta occurred at Cbeta-2 in both cell lines. There were no evident differences in the TCR-beta rearrangement patterns of these cell lines. The HTLV-I antigens p19, p24, pr53, p40^{ax}, and gp46 were detected in both WLeuk-1 and TARS-1 cells on enhanced chemiluminescence Western blot analysis (Fig. 4A and B). Expression of the HTLV-I core proteins p19, p24, and pr53 and that of the envelope protein gp46 were lower in WLeuk-1 than in TARS-1 cells, while p40^{ax} and p21^x production was about five times higher in WLeuk-1 cells than in TARS-1 cells. In contrast, the expression of p27^{ex} was similar in the two lines (Fig. 4C and D). The p27^{ex} protein content of MT-2 cells was extremely high. Expression of MHC class II in WLeuk-1 cells was reduced to about 1/4.6 times that in TARS-1 (Fig. 4C and 5), and then IL-2R-alpha expression was increased until it was about five

FIG. 4. Enhanced chemiluminescence Western blotting analyses of HTLV-I proteins. (A) Total HTLV-I proteins detected with an ATL patient's serum as the primary antibody; (B) expression of $p40^{tax}$ detected with Lt-4, a mouse monoclonal antibody to $p40^{tax}$; (C) expression of $p40^{tax}$ and MHC class II detected with the mouse monoclonal antibodies Lt-4 and MHC-OX6; (D) expression of $p27^{rex}$ and $p21^x$ detected with the rat monoclonal antibody Rec-6.

times stronger in WLeuk-1 cells than in TARS-1 cells (Fig. 5). The WLeuk-1 cell population tended toward lower production of MHC class II molecules, but MHC class I expression in WLeuk-1 was similar to that in TARS-1. The reproducibility of these analyses was confirmed by repeated experimentation.

Karyotype analyses of WLeuk-1 cells. Chromosome analyses of the TARS-1 and WLeuk-1 cell lines were performed with cells from the same passage as those subjected to Southern blot analysis. Figure 6A shows representative karyotypes of each cell line. The modal chromosome number of TARS-1 was 42, although the distribution of TARS-1 cell chromosome numbers was biphasic; there were two main populations with common marker chromosomes (Fig. 6). On the other hand, WLeuk-1 exhibited relatively homogeneous distribution, with a modal chromosome number of 42. As shown in Fig. 6, the origins of marker chromosomes (A through K) were partially traceable. Markers B1 and D were derived from a reciprocal translocation between chromosomes 2 and 4, and marker chromosomes I and J were unknown fragments characteristic of TARS-1 cells containing 44 chromosomes.

WLeuk-1 cells retained the same marker chromosomes, B1, D, E, and F, as TARS-1 cells and contained three specific marker chromosomes, M1, M2, and M3 (Fig. 7). M1 (3p+) was a chromosome 3 derivative with a chromosome 4 fragment on the short arm, M2 was derived from t(12;13), and M3 (Xq+) had an additional unidentified segment at the distal portion of the long arm of chromosome X and differed from marker chromosome K seen in TARS-1 cells.

DISCUSSION

In this study, TARS-1 cells were rejected by almost all of the newborn WKAH/Hkm rats; only 1 of 65 developed a leukemia/lymphoma-like disease. TARS-1 was initially reported to be transplantable to newborn WKA/Hok rats and to cause rapid deterioration in these animals (28). The behavior of TARS-1 cells in the WKAH/Hkm rats used in this study was quite different. The WKAH/Hkm rat is a substrain of the WKA strain, separated at F206 in 1974 from WKA/Hok and thereafter maintained by strict full-sib mating. At least 30 generations have passed since separation, and no genetic differences among them have been detected

FIG. 5. Fluorescence-activated cell sorter analysis of MHC class I, MHC class II, and IL-2R- α of both WLeuk-1 (solid jagged line) and TARS-1 (broken jagged line) cells. Cells were treated with MRC-OX18 (MHC class I), MRC-OX6 (MHC class II), or MRC-OX39 (IL-2R- α), followed by fluorescein isothiocyanate-conjugated rabbit anti-mouse Ig. The dotted line (. . .) depicts the fluorescence profile obtained from the cell analysis without each primary antibody.

so far. The difference in TARS-1 cell transplantability suggests that either some genetic locus of WKAH/Hkm changed during long-term maintenance, resulting in a slightly different immune reaction to TARS-1 cells, or a phenotypic alteration occurred in the TARS-1 cells.

The gait disturbance and hind-leg paralysis observed with two of the inoculated rats are intriguing in relation to the motor abnormalities seen with HTLV-I-associated myelopathy/tropical spastic paraparesis (7). However, as the cerebrospinal fluid of these rats could not be sampled and the symptoms could not be examined in detail, we do not know whether these disturbances were related to HTLV-I-associated myelopathy/tropical spastic paraparesis or the diagnostic criteria of this disease were met. The gait disturbance in rat 310 could not be attributed directly to a tumor nodule in the cerebellar meninges, as tumor cells had not invaded the parenchyma of the cerebellum. However, through compression, a space-occupying intracranial mass might have caused such symptoms without invasion. It is also possible that a small tumor nodule at the base of the leg produced a gait disturbance by impeding the blood flow. Since complete histologic studies, including spinal cord examinations, could

not be accomplished, it is impossible to draw firm conclusions about the sources of the neurologic signs in these two animals.

WLeuk-1 cells established from the peripheral blood lymphocytes of a rat with a leukemia/lymphoma-like disease contained several chromosome markers identical to those of TARS-1, indicating that the WLeuk-1 cells originated from TARS-1. This was also supported by Southern blot analysis which showed a similar HTLV-I integration pattern in both cell lines that was determined by using five different restriction enzymes along with the same surface marker expression. Moreover, the same patterns of TCR-beta chain rearrangement were detected in both cell lines. This is strong evidence of a common origin, as TCR rearrangement patterns are specific to T-cell clones. An additional band detected by *EcoRI* digestion in the short-term WLeuk-1 culture was not seen in TARS-1 cells, which suggests the possibility of viral transmission to other lymphocytes in vivo or in vitro, clonal expansion of a minor TARS-1 population in vivo, gene rearrangement involving the HTLV-I genome, or jumping of HTLV-I to another chromosomal locus. HTLV-I contains a retro-transposon structure similar to that of *Drosophila* copia or its P elements, bacterial *Tn3* and *Tn10* transposons, and *Saccharomyces cerevisiae* Ty elements (21). Various chromosome abnormalities have also been observed in ATL cells, particularly at the TCR locus (22). However, there have been no reports about HTLV-I jumping to another chromosomal locus or gene rearrangement involving the HTLV-I genome. Therefore, the probability of these events seems extremely low. To test for clonal expansion of a minor TARS-1 population, we used the Southern blot technique to analyze the HTLV-I integration pattern in scores of TARS-1 subclones and found the pattern identical to that of the parent TARS-1 cells (data not shown). However, this finding may not be sufficient to exclude this possibility altogether. Although there is no direct evidence, it seems more likely that HTLV-I was transmitted to other lymphocytes in vitro or in vivo and that this cell population disappeared during cultivation. The established T-lymphocyte cell line WLeuk-1, in which the additional *EcoRI* band mentioned above had disappeared and the HTLV-I integration pattern was identical to that of the TARS-1 line, is possibly a product of in vivo migration and infiltration, since cell growth was vigorous and the phenotype progressed in transplanted cells. The malignant phenotype of WLeuk-1 was associated with additional specific chromosome abnormalities, inhibited expression of the MHC class II gene, and aberrant expression of HTLV-I regulatory genes. These phenomena are possible causes of the progression of TARS-1 to the WLeuk-1 cells that induce leukemia/lymphoma-like diseases.

One reason why leukemia/lymphoma-like diseases developed, even though the incidence of disease was extremely low, may relate to a breakdown of host immune surveillance mechanisms. Most patients with ATL are susceptible to various opportunistic infections, which, in fact, are the main causes of death (4). Functional alterations with HTLV-I infection of CD4⁺ multifunctional T cells and virus-specific CD8⁺ cytotoxic T-cell clones were previously suggested to be one of the mechanisms underlying HTLV-I-mediated immunodeficiency (8). Neutrophilia, which is occasionally seen with ATL (35), may also relate to such phenomena as abnormal production of lymphokines or cytokines by HTLV-I-infected cells and/or various opportunistic infections. Another possibility involves genetic changes, including activation of oncogenes or inactivation of tumor suppress-

FIG. 6. Representative karyotypes of TARS-1 and WLeuk-1 cells Q-banded with quinacrine mustard and Hoechst 33258. (A) TARS-1 cells were composed of two main populations containing 42 chromosomes (top two rows) and 44 chromosomes (middle two rows). WLeuk-1 cells (bottom two rows) showed a sharp single peak in chromosome number distribution at 42. Marker chromosomes were labeled alphabetically from A to K. Horizontal and diagonal bars indicate breakpoints, and the vertical bar indicates the loss of a homologous chromosome. Specific abnormalities observed in WLeuk-1 cells (M1 to M3) are indicated by arrows. (B) Deducible origins of marker chromosomes. Bars beside the markers indicate the conserved regions or rearranged positions. The broken line indicates an unclear breakpoint. A, 1p+; B1, t(2;4?); B2, t(2;9); C, 4p+(der(4;17q)); D, t(2;4); E, 6q+(der(6;7?)); F, 8p+(der(8;17p)); G, 11p+; H, 18p+; K, Xq+. I and J are unknown fragments.

sor genes, genetic events that induced clear phenotypic differences between WLeuk-1 and TARS-1 cells in their transplantability to newborn WKAH/Hkm rats: WLeuk-1 killed the hosts, while TARS-1 was almost always rejected. DNA polymerase-beta is transinactivated by $p40^{tax}$ of HTLV-I (11). Interference with DNA repair by transrepression of DNA polymerase-beta by $p40^{tax}$ enhances the possibility of host chromosome damage, which is frequently observed with ATL (11). Specific chromosomal abnormalities (M1, M2, and M3) found in the WLeuk-1 cells strongly support this possibility and may partially explain the phenotypic progression. The M1(3p+) marker would be most closely related to progression, because the rat *c-abl* oncogene is located on 3q12 (26). The breakpoint of M1 is still unclear from the present cytogenetic data, but it would be very interesting to know whether the expression of this oncogene was modulated and whether the organization of this oncogene, including its structural and regulatory sequence, was altered. Other genes affected by the specific chromosome abnormalities M2 and M3 should also be studied. Hybrid-cell experiments with these cells will provide more information concerning whether inactivation of some tumor suppressor genes was involved in these phenotypic changes.

The expression of core and envelope proteins of HTLV-I in WLeuk-1 cells was slightly depressed, while that of the transacting factor $p40^{tax}$ was enhanced about fivefold in comparison with that in TARS-1 cells. These phenomena are abnormal, as it has been shown previously that $p40^{tax}$ transactivates the expression of both HTLV-I structural genes and such cellular genes as the IL-2 receptor alpha chain (IL-2R-alpha), IL-2, IL-3, IL-4, granulocyte macro-

phage-colony-stimulating factor, lymphotoxin, and *c-fos* (9, 15, 17, 30). Recently, Akayama et al. (3) reported that $p21^x$ of HTLV-I inhibited functions of $p27^{tax}$. Therefore, in WLeuk-1 cells, overexpression of $p21^x$ may also suppress $p27^{tax}$ functions, which inhibit the splicing of HTLV-I mRNA and prevent the accumulation of nonspliced or single-spliced messages coding structural proteins such as *gag* and *env* (10). Thus, mRNA of HTLV-I within WLeuk-1 cells may accumulate in the spliced form, inhibiting the expression of structural genes and enhancing the expression of $p40^{tax}$ in these cells. On the other hand, high production of HTLV-I virions in MT-2 cells is seemingly attributable to increased expression of $p27^{tax}$, which results in the accumulation of nonspliced or single-spliced mRNA of HTLV-I coding *gag* or *env* messages and causes heightened production of HTLV-I structural genes. Owing to the strong expression of IL-2R-alpha in WLeuk-1 cells, the transactivating function of overexpressed $p40^{tax}$ may not be affected by progression. Reduced expression of HTLV-I structural genes and MHC class II in WLeuk-1 cells may be greatly advantageous for escaping immunological surveillance because of the lower presentation of HTLV-I structural proteins' epitopes in a complexed form with MHC class II molecules in the cytoplasmic membrane. These results suggest that the aberrant expression of $p40^{tax}$ and $p21^x$ was associated with phenotypic progression in WLeuk-1 cells. It may be important to determine whether enhanced expression of the *tax1* and $p21^x$ genes is one of the causes of the increased oncogenicity or merely incidental to phenotypic progression. Transfection experiments with these viral regulatory genes will clarify these issues. Our results concern-

FIG. 7. Characteristic abnormalities found in WLeuk-1. Three specific markers (M1, M2, and M3) from three representative metaphases were arranged left to right. Conserved regions are indicated by bars, and derivative chromosomes are numbered.

ing such transfection experiments will be reported elsewhere in the near future.

Although studies with rats (28, 36, 38), rabbits (2, 12, 24), hamsters (1, 6), and monkeys (31, 34) have been conducted, little information on the mechanisms of multistep leukemogenesis in ATL is available. The rat system we have described will provide information on the molecular biology of leukemia and will be useful in analysis of the functional regulation of p40^{tax}, p27^{rex}, and p21^x proteins in HTLV-I-infected cells.

ACKNOWLEDGMENTS

We thank R. C. Gallo, National Cancer Institute; J. Hamuro, Ajinomoto Co.; N. Yamamoto, Tokyo Medical and Dental University; H. Yamamoto, Kochi Medical School; and Takeda Chemical Industry, Ltd., for kindly supplying pMT-2 plasmid, Jurkat cells, HUT102 cells, the probe for the constant region of the mouse TCR beta chain, and recombinant IL-2, respectively.

Our thanks are also extended to T. Mitani and N. Kanakuni, Central Laboratory, Kochi Medical School, for their technical assistance.

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