RAT LYMPHOID CELL LINES WITH HUMAN T CELL LEUKEMIA VIRUS PRODUCTION

I. Biological and Serological Characterization

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Type C retroviruses have been implicated in the etiology of naturally occurring leukemias and lymphomas in several animal species. The extensive search for a possible retroviral etiology of some human leukemias and lymphomas has led to the first isolation of a novel type C retrovirus, human T cell leukemia virus (HTLV), from cultured human neoplastic T cells (1–3).

Recently, a type C retrovirus (ATLV) has been isolated from the cultured cells of adult T cell leukemia (ATL), a unique lymphoproliferative disease of T cell origin that has been recognized in Japan (4–9). ATLV seems to be causally related to ATL and is a member of the HTLV group (10–15). HTLV has been reported to have immortalizing effects on normal human, simian, and rabbit lymphocytes (16–21). In this paper, we report that HTLV successfully infected and immortalized rat lymphoid cells of the spleen, lymph nodes, and thymus when cocultivated with 5-bromo-2'-deoxyuridine (BrdUrd)-treated ATL cells. Furthermore, two of these cell lines established in vitro were transformed and transplantable into newborn syngeneic rats as well as into nude mice. Adult rats that rejected these cell lines, after repeated immunizations, produced high titered antibodies against HTLV.

Materials and Methods

Animals. Inbred Wistar-King-Aptekman (WKA/Hok) rats were kindly supplied by Prof. M. Aizawa (Department of Pathology, Hokkaido University School of Medicine, Sapporo, Japan). Nude mice (BALB/c- nu/nu) were obtained from the Department of Genetics and Breeding, Central Institute for Experimental Animals, Tokyo, Japan.

Antisera. ATL patients' sera that contained high titered antibodies for ATL-associated antigen (ATLA) were used. Rabbit anti-Ra-1 serum (22) that contained specific antibodies for the structural proteins of HTLV was generously provided by Prof. I. Miyoshi (Department of Medicine, Kochi Medical College, Kochi, Japan). The monospecific...
antisera for structural proteins, gp70, p30, and p15(E) of murine type C virus (Rauscher), p30 of feline type C virus, and p30 of simian type C virus (SSV-1) were prepared in rabbits or in goats and generously provided by Drs. W. D. Hardy, Jr., E. Fleissner (Memorial Sloan-Kettering Cancer Center, New York), and J. T. August (Johns Hopkins University, School of Medicine, Baltimore, MD). The specificity of these antisera for immunofluorescence reaction was confirmed by the absorption of the antisera with either intact virus particles or purified viral proteins (23-25).

**Cells.** Fresh ATL cells were obtained from the heparinized blood of a 68-year old male ATL patient using the Ficoll-Paque (Pharmacia, Sweden) gradient. The patient had leukocyte counts of 106,700/mm³ of which >90% were ATL cells with indented or lobulated nuclei. Anti-ATLA titer in the serum was 1:1,280. The surface markers of leukemic cells were Leu-1(+), Leu-2a(−), Leu-3a(+), Leu-5(+), Leu-7(−), Leu-10(−), surface Ig(−), HLA-DR(−), and Leu-M1(−).

Cell suspensions of female WKA rats were prepared according to the method described elsewhere (26). After several washings, the cells were finally suspended in RPMI 1640 supplemented with 20% heat-inactivated fetal calf serum (FCS), penicillin (100 IU/ml), and streptomycin (100 µg/ml).

Tissue homogenates for absorption tests were prepared according to the method described elsewhere (23).

**Short-term Culture of ATL Cells in the Presence of 5-Bromo-2′-deoxyuridine (BrdUrd).** Fresh ATL cells were placed in tissue culture flasks (Corning, NY) at a density of 5 × 10⁶/ml in RPMI-1640 medium supplemented with 20% FCS containing 50 µg/ml of BrdUrd (Sigma Chemical Co., St. Louis, MO). They were incubated at 37°C for 3 d in a humidified 5% CO₂ atmosphere. The cells were washed twice with cold Hanks’ balanced salt solution and used for either cocultivation study or electron microscopic observation.

**Cocultivation.** First, spleen cells, lymph node cells, and thymocytes of female WKA rats were cultured at a density of 1 × 10⁶/ml in tissue culture flasks with RPMI-1640 supplemented with 20% FCS for 9 d. An equal volume of washed ATL cell suspension (1 × 10⁶/ml) pretreated with BrdUrd was then added to each culture flask. Control cultures included one culture flask each of the rat spleen cells, lymph node cells, thymocytes, and ATL cells pretreated with BrdUrd alone. All cultures were incubated at 37°C in a humidified 5% CO₂ atmosphere and fed twice a week.

**Cell Lines.** The HTLV-producer human cell lines MT-1 (6) and MT-2 (8) were kindly supplied by I. Miyoshi. The HTLV-negative cell lines Molt-4F and EB-Wa were kindly provided by Dr. H. Ikeda (Asahikawa Medical College, Asahikawa, Japan). The HTLV-producer rat lymphoid lines TARS-1, TARL-2, and TART-1, will be described in the text.

**Immunofluorescence (IF) Analysis.** Indirect IF tests of acetone-fixed cells for murine, feline, and simian type C viral proteins, and for ATLA were performed as previously described (7, 24). Anti-ATLA antibodies were titrated with acetone-fixed MT-2/Molt-4F cells. Briefly, one portion of ATLA-positive MT-2 cell suspension (1 × 10⁶/ml) and three portions of ATLA-negative Molt-4F cell suspension (1 × 10⁶/ml) were mixed and the cells were dropped onto multisport microscopic slides (C. A. Hendley Ltd., Essex, England). The slides were incubated overnight on wet filter paper in a large, clean petri dish. After a quick rinse with PBS and distilled water, the cells were fixed in cold acetone for 10 min and allowed to air dry. Selective IF staining of ~25% of the fixed cells was a positive indication for anti-ATLA antibodies.

Membrane IF tests for rat cell surface markers were kindly performed by Drs. Y. Ishii, A. Matsuura, and Prof. K. Kikuchi, Department of Pathology, Sapporo Medical College, Sapporo, Japan, as described elsewhere (27).

**Absorption Test.** The specificity of IF reactions with rat anti–TARL-2 serum (see text) was confirmed by absorption tests with cell lines, rat cells, and homogenates. The method for absorption was described elsewhere (23).

**Transplantability of Rat Cell Lines into Newborn Syngeneic Rats and Nude Mice.** Newborn WKA rats were injected intraperitoneally with either viable TARS-1, TARL-2, or TART-1 cells in doses of 1 × 10⁷ cells. Rats were examined at 2 wk for histology. Nude mice
were inoculated subcutaneously with either viable TARS-1, TARL-2, or TART-1 cells in doses of 1 × 10^7 cells.

**Immunization of Adult Syngeneic Rats with Rat Cell Lines.** Adult WKA rats were injected intraperitoneally with either TARS-1 or TARL-2 cells in doses of 1 × 10^7 cells, followed by an intravenous injection into tail vein at the same dosage at 1-wk intervals. After being injected five times, the rats were bled to death. The sera were collected and stored at −70°C until use.

**Labeling of Cells and SDS-PAGE Analysis.** Labeling of MT-2 cells with metabolic precursor, [35S]methionine (Amersham Co. Ltd.), was carried out according to the method described (28). The labeled cell suspension was washed three times with phosphate-buffered saline and the cells were lysed with RIPA lysis buffer (0.15 M NaCl; 0.5 mM MgCl₂, 0.6 M KCl, 1 mM phenylmethylsulfonylfluoride, 10,000 KU/100 ml Trasylol Aprotinin, 10 mM Tris-HCl, pH 7.8) containing 2% Triton X-100. The soluble extracts were cleared by centrifugation at 25,000 g for 15 min at 4°C. Before precipitation of viral immunoreactive proteins, the soluble extracts were incubated with anti-ATLA negative human or rat serum for 16 h at 4°C at a ratio of 35 μl of serum to 100 μl of cell extract. The IgG in the mixture was removed with protein A-Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ). After centrifugation, the supernatant was then incubated for 4 h at 4°C with 20 μl of various sera with or without antibodies against HTLV structural proteins. After further incubation with protein A for 45 min at 4°C, the immune complexes were precipitated by centrifugation. They were washed twice and resuspended in 70 μl of electrophoresis sample buffer (0.0625 M Tris-HCl, pH 6.8, 2% SDS, 4% mercaptoethanol) and heated for 3 min in a boiling water bath. After addition of 10% glycerol and 0.05% bromophenol blue, the samples were electrophoresed in SDS-polyacrylamide slab gels and prepared for fluorography. A sample containing 14C-labeled bovine serum albumin (mol wt 69,000), ovalbumin (mol wt 46,000), carbonic anhydrase (mol wt 30,000), and lysozyme (mol wt 14,300) was simultaneously run as a molecular weight marker (Amersham Co. Ltd.).

**Electron Microscopy.** For electron microscopy, cell pellets and tissues were fixed in 2% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate, followed by lead nitrate, and examined in a Hitachi H-600 electron microscope.

**Cytogenetic Analysis.** Cytogenetic studies were performed on rat cell lines without phytohemagglutinin. The cells were incubated in the presence of colcemid, 0.02 μg/ml, for 3 h at 37°C, and then treated with hypotonic solution for 15 min at room temperature, and fixed in methanol-acetic acid (3:1). Chromosome preparations were made using flame-drying techniques. Chromosomes were stained with conventional Giemsa and analyzed under usual light microscopy.

**Light Microscopy.** The cells were stained with May-Grünwald-Giemsa and periodic-acid/Schiff (PAS) and for α-naphthyl acetate esterase and acid-phosphatase activity by the usual cytochemical techniques. Tissues of WKA rats were fixed in 10% formalin. Four stains were used: hematoxylin and eosin, periodic acid Schiff (PAS), silver impregnation, and methyl green pyronin.

**Results**

**Establishment of Rat Lymphoid Cell Lines by Superinfection with HTLV.** 1 wk after the cocultivation of female rat spleen cells with BrdUrd-treated ATL cells, small clumps of cells began to appear and gradually increased in size and number. After two months, the cells began to proliferate vigorously in suspension forming large clumps of cells, and have since been maintained in continuous culture for over 8 months. This cell line was designated TARS-1. The TARS-1 cells grown in suspension had a tendency to clamp and to adhere to the plastic. The cells were generally round or ovoid and varied in size with a modest amount of agranular cytoplasm. The nuclei were mostly oval and nucleoli were indistinct.
The modal cell diameter was 12 μm. The majority of cells were PAS reaction negative, but occasional cells showed granular PAS positivity in the cytoplasm. The cells were uniformly and intensely stained for acid phosphatase, which appeared as paranuclear large roundish clumps, indicating that TARS-1 is a rather homogeneous population (Fig. 1A). The cells were also weakly positive for α-naphthyl acetate esterase. Cytogenetic analysis of TARS-1 showed a female rat karyotype with 42 chromosomes (Fig. 1B).

Subsequently, we succeeded in establishing two rat lymphoid cell lines, TARL-2 and TART-1, which originated in the lymph node and thymus, respectively. Both cell lines grew in suspension and formed clumps without a tendency to adhere to the plastic. Both cell lines were uniformly and intensely stained for acid phosphatase, which appeared as paranuclear large roundish clumps. Cytogenetic analysis of TARL-2 and TART-1 showed a female rat karyotype with 42 chromosomes. Rat spleen cells, lymph node cells, thymocytes, or pretreated ATL cells alone did not grow and degenerated within 2 months.

**Cell Surface Markers.** The results of surface marker tests, which were used to characterize rat and human lymphocytes, are summarized in Table I. TARS-1 cells were positive for rat Thy-1, Ly-1, Ia, and leukocyte common antigens, but negative for rat Ly-2, surface Ig, and human Leu-1 antigens. TART-1 cells were positive for rat Thy-1, Ia, and leukocyte common antigens, but negative for rat Ly-1, Ly-2, surface Ig, and human Leu-1 antigens. These results indicate that TARS-1 and TART-1 originate in rat T cells.

TARL-2 cells were positive for rat Ia and leukocyte common antigens, but negative for rat Thy-1, Ly-1, Ly-2, surface Ig, and human Leu-1 antigens.

**Expression of Type C Viral Antigens.** Fixed cells of TARS-1, TARL-2, and TART-1 were examined for the expression of various type C retrovirus antigens with indirect IF. Three cell lines were only positive for ATLA demonstrated by both human sera containing anti-ATLA antibody and rabbit anti-Ra-1 serum. ~20% of the fixed cells were brightly stained for ATLA in the cytoplasm with granular pattern (Fig. 1C). Tests for the antigens related to murine gp70, p30, p15(E), feline p30, and SSV-1 p30 were negative in all three cell lines, indicating the unlikelihood of activation of rat endogenous type C viruses which have antigens shared with murine, feline, and simian type C retroviruses.

**Electron Microscopy.** After short-term culturing with BrdUrd, ATL cells produced type C virus particles morphologically identical to HTLV. Large numbers of mature and immature particles were found in the extracellular space in clumps associated with cellular debris. Budding from the cell membrane was also seen.

TARS-1 cells, sized 8–15 μm in diameter, had a rather uniform oval nucleus. Special configurations of the nuclear envelope, such as irregular deep indentation or lobulation, were not seen. The chromatin was rather evenly distributed, and the nucleoli were prominent. The cell membrane was smooth and the cytoplasm was relatively abundant and rich in membranous organelles and in polyribosomes. Extracellular type C virus particles were occasionally seen. Budding form was practically absent. After short-term culturing with BrdUrd, TARS-1 cells produced a considerable number of mature and immature type C virus particles morphologically identical to HTLV in the extracellular space (Fig. 1D). Occa-
FIGURE 1. Cytologic, cytogenetic, immunofluorescent, and ultrastructural characteristics of TARS-1. (A) TARS-1 cells uniformly contain large roundish clumps of acid phosphatase activity in the cytoplasm (X 1,000). (B) Cytogenetic analysis of TARS-1 showing a normal female rat karyotype with 42 chromosomes. (C) Detection of ATLA in acetone-fixed TARS-1 cells by indirect immunofluorescence. The cells were first treated with ATL patient's serum at a dilution of 1:20 which contained an anti-ATLA antibody titer of 1:1,280 followed by fluorescein-conjugated anti-human IgG. Note granular fluorescence in the cytoplasm (X 200). (D) Electron micrograph of TARS-1 treated with BrdUrd. Mature and immature type C virus particles are seen in the extracellular space associated with cellular debris (Bar, 200 nm).
HUMAN RETROVIRUS PRODUCER RAT CELL LINES

Table I

| Cell line | Rat markers | Human markers |
|-----------|-------------|---------------|
|           | Thy-1 | Ly-1 | Ly-2 | Ia | Leukocyte common | slg | Leu-1 |
| TARS-1    | +    | +    | -    | +  | +                | -   | -     |
| TARL-2    | -    | -    | -    | +  | +                | -   | -     |
| TART-1    | +    | -    | -    | +  | +                | -   | -     |

Sionally, typical type C viruses were observed budding from the cell membrane. The other two cell lines showed a similar morphology with virus production.

Transplantability of TARS-1 and TART-1 but Not TARL-2 Cells into Newborn Syngeneic Rats and Nude Mice. To determine whether rat cell lines established in vitro are associated with cell transformation, newborn syngeneic WKA rats were injected intraperitoneally with either TARS-1, TARL-2, or TART-1 cells. After 2 wk, all the rats injected with TARS-1 rapidly deteriorated. The rats were killed, and the organs were examined histologically. Out of the nine rats all showed marked swelling of the mediastinal and mesenteric lymph nodes. The paraaortic and the hilar lymph nodes of the spleen were also swollen. The tumor nodules were frequently seen at the injection site. Whitish nodules were disseminated in the parenchyma of the liver and lung. The spleen was slightly enlarged. The thymus, bone marrow, and other organs appeared intact. By light microscopy, lymph nodes of the mediastinum, mesenterium, paraaorta, and splenic hilus were totally replaced by a massive infiltration of large lymphoblastic cells morphologically similar to TARS-1. The subcutaneous nodules at the injection site showed a diffuse proliferation of tumor cells with a densely packed arrangement. The lungs also showed tumor growth, which formed multiple nodules in the parenchyma (Fig. 2). The tumor cells infiltrated mainly along the portal tract, forming occasional tumor nodules in the liver. The spleen showed microscopic tumor infiltration. The tumor cells infiltrated diffusely into the omentum and pancreas. The thymus and other organs were negative for tumor infiltration. TARS-1 cells were not transplantable into adult WKA rats. TARS-1 inoculated subcutaneously in nude mice grew slowly forming nodules. After 2 months, the nodule measured 2.0 × 1.5 × 1.5 cm. The histological examination showed massive subcutaneous growth of tumor cells resembling TARS-1. TART-1 was also transplantable into newborn syngeneic rats and nude mice with similar growth patterns. In contrast, TARL-2 cells were transplantable into neither newborn and adult WKA rats nor nude mice. It was concluded that TARS-1 and TART-1 are cell lines with tumorigenic activity in vivo.

Production of Specific Antibodies for HTLV in Adult WKA Rats Immunized with Rat Cell Lines. Since TARS-1 and TARL-2 cells were not transplantable into adult rats, WKA rats were given i.v. injections of either TARS-1 or TARL-2 cells at 1-wk intervals. The sera obtained from hyperimmune rats with TARS-1 cells contained antibodies for ATLA with a titer of 1:640 as seen by IF. Rats immunized with TARL-2 cells also produced high titered antibodies for ATLA
FIGURE 2. TARS-1 transplanted into a newborn rat. The tumor infiltrates diffusely in the pulmonary parenchyma forming multiple nodules (H&E, × 100).

**TABLE II**

*IF Specificity of Antigens Demonstrated with Rat Anti-TARL-2 Serum*

| Antigens used for absorption | Residual antibody activity* on fixed MT-2/Molt-4F cells | Result of absorption |
|-----------------------------|--------------------------------------------------------|---------------------|
| WKA rat                     |                                                        |                     |
| Thymus                      | ++                                                     | −                   |
| Spleen                      | ++                                                     | −                   |
| Lymph node                  | ++                                                     | −                   |
| Liver                       | ++                                                     | −                   |
| Kidney                      | ++                                                     | −                   |
| Brain                       | ++                                                     | −                   |
| Erythrocytes                | ++                                                     | −                   |
| Cultured cells              |                                                        |                     |
| MT-2                        | −                                                      | +                   |
| MT-1                        | −                                                      | +                   |
| EB-Wa                       | ++                                                     | −                   |
| Molt-4F                     | ++                                                     | −                   |
| TARS-1                      | −                                                      | +                   |
| TARL-2                      | −                                                      | +                   |
| Fetal calf serum            | ++                                                     | −                   |

* Rat anti-TARL-2 serum was used at a serum dilution of 1:250. The residual antibody activity was determined by immunofluorescence (IF).

with a titer of 1:1,280. In order to demonstrate the antibody specificity, rat antisera were absorbed with various tissues and cultured cells listed in Table II. The absorbed serum was tested for residual antibody activity on fixed MT-2/
Molt-4F cells by indirect IF. Cultured cell lines producing HTLV, MT-2, MT-1, TARS-1, and TARL-2 absorbed the antibody activity. HTLV nonproducer cell lines, Molt-4F and EB-Wa, did not absorb the antibody activity. Neither normal rat cells and tissues nor FCS absorbed the antibody activity. These findings strongly indicate that the antigen(s) recognized by rat antisera is specifically related to HTLV.

Structural Proteins of HTLV Demonstrated by Rat Antisera. Cytoplasmic extracts of MT-2 cells labeled with [35S]methionine were allowed to react with rat anti-TARL-2 serum and anti-ATLA-positive human serum. Anti-ATLA-negative human and rat sera were included as negative controls. The antigen-antibody complexes were adsorbed onto protein A-Sepharose 4B and then the samples were redissolved and analyzed in 10% SDS PAGE and autoradiography.

Two polypeptide bands were specifically precipitated with rat anti-TARL-2 and anti-ATLA-positive human serum. These polypeptides have molecular weights of approximately 28,000 (p28) and 24,000 (p24) daltons (Fig. 3). P24 appears to be the major polypeptide demonstrated by both rat anti-TARL-2 serum and anti-ATLA-positive human serum.

Discussion

The present study confirms and expands on the previous findings of Miyoshi et al. and others (16–21) that HTLV has a wide range of host animals in that it is transmissible not only to human and primate lymphocytes, but also to rabbit lymphocytes in vitro. In our study, HTLV preferentially infected and transformed rat T cells of the spleen and thymus. The exact character of TARL-2, which originated in the lymph nodes, was not determined since TARL-2 cells were only positive for rat la and leukocyte common antigens. Nevertheless, the present study suggests a tenacious T cell-tropism of HTLV in a wide range of

Figure 3. SDS-PAGE analysis of the immunoprecipitates formed between [35S]-labeled MT-2 polypeptides and normal rat serum (A), rat anti-TARL-2 serum (B), ATL patient serum (C), and normal human serum (D).
host animals. Because HTLV is not endogenous in humans (3, 9), a more complete host range study or an analysis of whether or not wild rats are naturally infected with HTLV in ATL endemic areas would be of considerable interest.

It was demonstrated that the ability of TARS-1, TARL-2, and TART-1 to be transplanted in vivo differs. TARS-1 and TART-1 cells were transplantable not only in newborn syngeneic rats but also in nude mice, whereas, TARL-2 cells did not grow in either newborn syngeneic rats or in nude mice. The evidence suggests that TARS-1 and TART-1 are cell lines that have been transformed by HTLV. Although the complete nucleotide sequence of the provirus genome has been fully determined, the location or even the existence of the transforming gene(s) of HTLV is not clear at the present time (29). Nevertheless, the present study strongly indicates that HTLV not only immortalizes, but also transforms rat T cells in vitro. Its effect on rat T cells suggests either that HTLV contains a transforming gene or that it activates rat cellular onc. Studies are under way to clarify these points.

Several researchers have reported the presence of natural antibodies in human sera that reacted with the structural proteins of HTLV (7, 9-12, 14, 15, 28). The major reactive antigens they reacted with were internal structural proteins with molecular weights of 24,000 daltons (p24) and 19,000 daltons (p19) (9-12, 14, 15, 28). WKA rats immunized with either TARS-1 or TARL-2 produced antibodies specific for HTLV-producer cell line, MT-2. The biochemical analysis of antigens that reacted with rat antisera indicated that molecular species bearing antigenic determinants are at least two HTLV-related polypeptides, p24 and p28. P24 seems to be the major core polypeptide of HTLV. Recently, Tanaka et al. (30) have demonstrated, using a mouse monoclonal antibody that reacts with both HTLV-specific polypeptides p19 and p28, that both p19 and p28 were found in MT-2 and T cell lines newly established by superinfection with MT-2, whereas only p19 but not p28 was found in MT-2 unrelated HTLV producer cell lines. They suggested that HTLV-producer cell lines can be classified into two groups according to the presence or absence of p28 (30), and combining this with the fact that attempted transformations of normal cells by the superinfection with p28-defective HTLV producers have failed, they presumed that the expression of p28 may have an important function in the in vitro transformation of normal cells by HTLV. This may be consistent with the recent finding that the nucleotide sequence of the HTLV provirus contains four open reading frames other than those for gag, pol, and env genes. One of them could code for 27,000-dalton polypeptide (29). Determination of whether or not p28 that was detected by rat sera as well as human sera containing anti-ATLA antibody, is coded by the HTLV genome or related to cell transformation will require further research.

Finally, the establishment of the three rat cell lines with HTLV production reported here, TARS-1, TARL-2, and TART-1 may provide biologically and genetically useful information on HTLV and the molecular mechanism of its action on T cells.
Cocultivation of spleen cells, lymph node cells, and thymocytes of female Wistar-King-Aptekman rats with short-term cultured male adult T cell leukemia (ATL) cells in the presence of 5-bromo-2'-deoxyuridine (BrdUrd) resulted in the establishment of rat lymphoid cell lines, TARS-1, TARL-2, and TART-1. Cytogenetic analysis of the three cell lines showed a female rat karyotype with 42 chromosomes. The surface phenotypes of TARS-1 and TART-1 were those of rat T cells. TARL-2 was only positive for rat Ia and leukocyte common antigens. The cell lines continuously produced a type C retrovirus, human T cell leukemia virus (HTLV) and expressed ATL-associated antigens. TARS-1 and TART-1, but not TARL-2 were transplantable into newborn syngeneic rats and nude mice. These results strongly indicate that HTLV not only immortalizes, but also transforms rat T cells in vitro. Adult rats immunized with either TARS-1 or TARL-2 produced antibodies specific for HTLV. The biochemical analysis of the antigens that reacted with rat sera revealed that they are the two HTLV-specific polypeptides, p24 and p28.

The authors would like to extend their sincere thanks to Doctors M. Aizawa, I. Miyoshi, W. D. Hardy, Jr., E. Fleissner, J. T. August, C. Mikuni, Y. Ono, and M. Miyata for their generous supply of rats, antisera, and ATL cells, and to Y. Ishii, A. Matsuura, and K. Kikuchi for their efforts in performing examinations for us of rat cell surface markers. We are also grateful to Prof. S. Kuzumaki of the Laboratory of Genetics, Cancer Institute, Hokkaido University School of Medicine, for his invaluable advice, and to Mr. K. Kobayashi, M. Taniguchi, A. Nakamura, M. Nozaki, N. Mizuno, and Ms. A. Mita for their technical and secretarial assistance.

Received for publication 12 December 1983.

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