Specific Adsorption of HTLV-I to Various Target Human and Animal Cells

By Karen Krichbaum-Stenger, Bernard J. Poiesz, Paul Keller, Garth Ehrlich, Jerrie Gavalchin, Bruce H. Davis, and Janet L. Moore

In this report, we describe a flow cytometric analysis of HTLV-I specific binding to fresh and cultured cells on a single cell basis. This assay uses rhodamine hydrocarbon tagged, purified HTLV-I virions according to the procedure originally described for avian retroviruses. Successful HTLV-I transmission was detected by analysis of integrated HTLV-I DNA, virion-associated reverse transcriptase, and/or intracellular HTLV-I core antigen p19 expression. Only a specific virus–cell interaction was detected because nonrhodamine-tagged homologous virus or related HTLV-II interfered with tagged HTLV-I binding. In contrast, an unrelated, nonlabeled animal retrovirus was unable to block tagged HTLV binding. Of the cell lines tested, 2 nonlymphoid mammalian and 3 human lymphoid bound significantly high to moderate levels of HTLV-I–tagged virions. The other three human lymphocyte cell lines were insensitive to HTLV-I adsorption. A direct correlation was observed between HTLV-I binding sites and infectivity of human lymphoid cells alone and not other nonlymphoid animal cells. Fresh normal human mononuclear cells bound low levels of HTLV-I virions. As expected, T lymphocytes demonstrated more binding than did the non-T cell population. Enhancement of HTLV-I cell binding in a subpopulation of mononuclear target cells was achieved with phytohemagglutinin (PHA) activation and interleukin 2 (IL2) stimulation, which correlates well with previously published infectivity studies.

The human T cell lymphoma/leukemia virus I and II are associated with adult T cell leukemia (ATL) and T cell hairy cell leukemia, respectively. Functionally, both viruses transform T lymphocytes in vitro. In vitro infection of a wide range of human and animal cells by both viruses has been reported. Using cocultivation techniques, normal, T4, T8, and non-B/non-T lymphocytes can be infected. Lymphocytes from several other species can also be infected. Human-derived cell lines, including B lymphocytes, osteogenic sarcoma cells, fibrosarcoma cells, diploid fibroblasts, and endothelial cells can also be infected.

The only nonhuman, nonlymphoid cell line capable of HTLV-I transmission is feline kidney fibroblasts. The in vitro host range of HTLV-II differs slightly from HTLV-I. Although equally transmissible to human T lymphocytes, HTLV-II more efficiently infects B lymphocytes and is not transmitted to human fibrosarcoma, human osteosarcoma, or feline kidney fibroblasts.

Despite their varied in vitro tropism, however, HTLV-I and HTLV-II have been associated only with human malignancies exhibiting the T4, T8, and non-B/non-T lymphocytes cell surface phenotype. This in vivo cellular tropism of HTLV-I may be regulated at several points in the virus life cycle: (a) viral adsorption and penetration, governed by specific viral target membrane receptors; (b) viral genomic integration into target cell DNA; and (c) RNA transcription of proviral DNA, requiring noncoding, viral-specific, regulatory sequences called long terminal repeats (LTRs).

Several lines of evidence support the concept that the cell surface receptors may be a key in the determination of HTLV-I and HTLV-II cell tropism in vivo. First, although the major envelope glycoproteins of HTLV-I and HTLV-II are distinct, they are more homologous than the LTR transcriptional regulatory sequences of HTLV-I and HTLV-II. In addition, by vesicular stomatitis virus (VSV) pseudotype interference studies, these two related human retroviruses appeared to share the same cell membrane receptors. Another argument involves the lack of site-specific integration of HTLV-I into cellular chromosomal DNA. Examination of HTLV-I proviral DNA integration sites in fresh and cultured cells has indicated that the virus does not integrate in any preferential site or chromosome.

This virus does not appear to directly regulate RNA transcription of an adjacent cellular gene by cis activation. Last, recent data suggest that the molecular mechanisms of HTLV-I transformation involves trans activation of HTLV-I expression itself and possibly a yet unknown, oncogenic gene at a distant chromosomal location by the HTLV-I pX protein, LOR. These observations suggest that HTLV-induced cell transformation is primarily dependent on the susceptibility to virus attack at the specific cell membrane receptor level. Accordingly, the present studies focus on the development of an assay for relative quantitation of HTLV-I and HTLV-II cell membrane binding sites by flow cytometric analysis of rhodamine hydrocarbon-labeled HTLV bound to fresh and cultured cells on a cell–cell basis, as previously described for avian retroviruses.

MATERIALS AND METHODS

Cells. The following cell lines were maintained in culture: HUT 102-B2, an HTLV-I–producing, mature neoplastic T lymphocyte from a lymph node of an ATL patient; MT-2, umbilical cord blood mature T lymphocyte spontaneously infected with HTLV-I; Mo-T, HTLV-II–producing, human T lymphocyte from F. Ruscetti, National Institutes of Health (NIH); and the virus negative lines;

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Molt-4, an immature T lymphocyte derived from a patient with acute lymphocytic leukemia; HUT 78, mature T lymphocytes derived from a patient with cutaneous T cell lymphoma; FR-45, continuous human B lymphocytes from a normal donor from F. Ruscetti, NIH; NK-41, continuous human natural killer cells from a normal donor established by F. Ruscetti, NIH; VERO, an African green monkey kidney cells; and SC-1, a wild mouse fibroblasts from C. Kozak, NIH. Normal adult peripheral blood mononuclear cells were obtained by centrifugation of heparinized blood on a Ficoll-Hypaque density gradient. Activated T cells were generated by culturing the above cells in RPMI 1640 media, 10% fetal calf serum (FCS), 5 μg/mL phytohemagglutinin (PHA), and 10% dectinated, partially purified human interleukin 2 (IL 2) (Cellular Products, Buffalo).

T cell and non-T cell-enriched population were prepared by forming E rosettes with treated sheep RBCs (SRBCs) and performing sedimentation on lymphocyte separation media (Litton Bionetics, Kensington, MD).

Purification of retrovirus particles. Virions of HTLV-I, HTLV-II, or a B tropic, ecotropic murine leukemia virus (255T) from a BALB/c mouse were purified from media conditioned by the following cell lines; HUT 102-B2, Mo-T, and SC-1, respectively. To prevent possible disruption of intact whole virions or removal of their envelope proteins, a gentler purification was performed in comparison to previously described more stringent virus purification techniques. After removal of cultured cells from 2 to 20 L growth media (RPMI 1640, 10% FCS, 1% penicillin-streptomycin by sedimentation at 870 g for 15 minutes, virions were concentrated by centrifugation of the supernatant at 30,500 g for 45 minutes and resuspended in 10 mmol/L Tris-HCl, pH 7.4, 0.1 mol/L NaCl, 1 mmol/L EDTA (TNE buffer). Pooled virions were then spun through 30% sucrose in TNE buffer onto a 45% sucrose cushion by ultracentrifugation on two consecutive discontinuous 30% to 45% sucrose density gradients at 89,400 g for 2.5 hours. Virions were stored in 200–μL aliquots at −70°C.

Reverse transcriptase assay. Virus particles were quantitated according to the amount of reverse transcriptase activity present per lot of purified virions. Assessment of reverse transcriptase activity was carried out as previously described. Results were expressed in values of pmoles of 3H-deoxyxyunadine monophosphate incorporated per hour.

Labeling of virus with a fluorescent membrane probe. Rhodamine-16 (R-16) is a fluorescent dye (rhodamine) covalently linked to a saturated hydrocarbon 16 carbons long. The synthesis of similar membrane probes is described in ref. 23. The hydrocarbon chain causes the fluorescent dye to partition into lipid-rich viral envelopes and to remain there preferentially. The labeling technique that maintains infectivity of the virus and target cell viability was established previously. In brief, virions were conjugated with rhodamine-16 (HTLV-R-16) by incubating gradient purified virus in a final concentration of 0.25 mmol/L R-16 in 5% dimethyl sulfoxide. Unbound R-16 and any free R-16 were removed by two consecutive discontinuous sucrose velocity gradient centrifugations. The virus was concentrated on a glycerol cushion, and the buffer above the cushion was analyzed for the presence of any remaining free R-16.

Sample preparation for flow cytometric analysis. The adsorption procedure of R-16--labeled virions to various cells was performed as follows: Various concentrations (1 HTLV-I/R16 concentration refers to the equivalent number of virions containing 50 pmol of reverse transcriptase activity) of R-16--labeled virions in glycerol and TNE buffer were incubated with 7.5 x 10^3 target cells in suspension in 0.2 mL minimal essential medium (MEM), 1% FCS and 2 μg/mL polybrene at 4°C, or room temperature. At 4°C, adsorption of HTLV-I occurs without subsequent penetration, whereas both viral adsorption and penetration of the target cell are possible at 25°C. After the virus–cell interaction, cells were spun at 400 g for 5 minutes at 4°C, supernatant was removed, and cells were resuspended in 0.4 mL fresh medium (MEM, 1% FCS, and polybrene). Samples were filtered through 44-μm nylon mesh to remove clumps and were kept on ice during flow cytometric analysis to maintain cell viability and to prevent significant viral penetration.

Multiparameter flow cytometric analysis. Flow cytometric (FCM) measurements were performed on the Coulter EPICS V (Coulter Electronics, Hialeah, FL) equipped with a 4-W argon laser. FCM measurements (relative fluorescent intensities) of rhodamine-labeled HTLV-I adsorbed cells resulted from excitation by the 514-nm line of the cytometer’s argon-ion laser, and the fluorescence emitted was monitored by detectors at wavelengths >610 nm using 530-nm short-pass interference and 570-nm long-pass absorbance filters. Data were expressed in histogram form. Each histogram is based on 10,000 events fitting the forward angle and 90° light-scatter gates to reduce the contribution of dead or clumped cells; the rhodamine fluorescence was quantitated. Each sample was screened for background fluorescence representing autofluorescence of cells in the absence of rhodamine. The distribution of fluorescence- positive cells was expressed on the y axis and the increasing intensity of fluorescence was expressed on the x axis on a log scale. The mean channel fluorescence data for all samples was converted to relative linear fluorescence values using a log to linear conversion method to allow better assessment of experiments.

Competitive inhibition studies using HTLV. Binding studies for the competition analysis were carried out as described above, except that prior to the addition of rhodamine-tagged virions, cells were incubated for 1/2 to 2 hours with varying amounts of unlabeled HTLV-I, HTLV-II, and/or murine leukemia virus (MLV) (as determined by relative reverse transcriptase activities).

HTLV transmission assay. Cocultivation of HTLV-I–positive donor, neoplastic T cells with various human lymphoid target cells was carried out as described. Resultant cell lines were assessed for HTLV-I production by assaying for extracellular reverse transcriptase and intracellular HTLV-I p19 core protein. The presence of integrated HTLV-I proviral DNA in the transformed cells was determined by dot blot or Southern blot hybridization using an HTLV-I cloned DNA probe.

HTLV p19 detection. Indirect immunofluorescent assays for HTLV-I p19 using an anti-HTLV-I p19 monoclonal antibody were performed by a previously described technique.

Nucleic acid hybridization. High-mol-wt DNA was extracted from the target, donor, and cocultivated cells and transferred onto nitrocellulose filters as previously described. Dot blot or Southern blot hybridization analysis was performed with these nitrocellulose filters and a radiolabeled HTLV-I gag-pol DNA sequence, pATK 32 (a gift of Dr M. Yoshida).

RESULTS

Binding site saturation. Figure 1 shows an HTLV-I/R-16 MT-2 cell binding saturation curve, established with increasing concentrations of gently purified HTLV-I labeled with R-16 and a constant number of target cells and time of adsorption. The observed relative mean fluorescence increased in a linear manner at the lower concentrations of HTLV-I/R-16. The plateau of virus adsorption was reached at an HTLV-I/R-16 inoculum containing 25 pmoles of reverse transcriptase, indicating significant saturation of binding sites. For this reason, the concentration of HTLV-I/R-16 chosen to bind 7.5 x 10^3 target cells in a standard
HTLV-I CELL SURFACE RECEPTOR

assay was 50 pmol of reverse transcriptase containing HTLV-I/R-16.

HTLV-I cell membrane binding assay. In all experiments, viable and nonviable cells were simultaneously analyzed by using forward-angle and 90° light-scatter parameters, but only the R-2 signals from 10,000 viable cells were measured. Figure 2 illustrates characteristic binding curves of free R-16 and R-16-labeled HTLV-I binding, respectively, to the prototype HTLV-I-producing cell line HUT 102-B2. For each cell population examined, the mean fluorescence bound per cell was recorded and the autofluorescence (fluorescence of cells in the absence of rhodinated virus) was checked. The HUT 102-B2-bound HTLV-I/R-16 histogram (Fig 2D) demonstrates significant adsorption of R-16-labeled virions to HUT 102-B2 cells with the maximum number of cells being represented by a mean fluorescence of 30, whereas the background autofluorescence of the same cells was minuscule (Fig 2A). The amount of fluorescence diminishes twofold (mean fluorescence 15) when target cells are incubated with unlabeled HTLV-I prior to the addition of rhodamine-tagged virions (Fig 2C). To rule out binding of residual free R-16 to HUT 102-B2 cells, HTLV-I/R-16 virus preparations were centrifuged to remove the labeled virions, and the supernatant (free of virions) was then reconstituted with HUT 102-B2 cells (Fig 2B). Results indicate that the HTLV-I/R-16 preparations are minimally contaminated with unbound R-16 or free rhodamine (Fig 2B).

MT-2 target cells infected with HTLV-I were chosen for reproducibility studies. Multiple tests on the same sample of HTLV-I/R-16 adsorbed MT-2 cells (instrument variability) and between replicate samples of HTLV-I/R16–bound MT-2 cells (assay variability) at near saturation showed a high degree of reproducibility. The SEM was 1.35% for instrument variability and 2.65% for assay variability (data not shown).

Homologous HTLV competitive binding. Figure 3 shows the histograms of relative HTLV-I/R-16 adsorption to several target cells and the effects of homologous virus competition. HTLV-I/R-16 adsorption specificity was clearly indicated in 57% and 52% of viable HUT 102-B2 and VERO cells, respectively (shaded areas). In contrast, only 28% of the normal adult peripheral blood mononuclear cells (PBMs) with PHA activation and IL 2 scored positive for HTLV-I/R-16 binding sites. These percentages are minimal numbers representing the number of HTLV-I/R-16–bound cells with a fluorescent intensity greater than the fluorescent intensity of tagged virion-bound cells that competed with homologous virus. The VERO cells expressed the highest number of specific fluorescent binding sites, more than twofold and fourfold higher than HUT 102-B2 and normal nonactivated adult PBMs, respectively (Fig 3B). Likewise, HUT 102-B2 cells showed an almost twofold greater rhodamine fluorescence over the PBMs tested (Fig 3A). The level of fluorescent intensity correlates with the surface area of these cells (data not shown). Homologous HTLV-I binding competition, with equal inoculums of unlabeled and labeled HTLV-I, reduced membrane bound fluorescence twofold in both the VERO and HUT 102-B2 cells. A concentration of unlabeled HTLV-I tenfold greater than HTLV-I/R-16 almost completely inhibited binding to HUT 102-B2 cells (data not shown). The activated adult PBM
cells were more resistant to competition with unlabeled homologous HTLV-I (Fig 3C).

**Kinetics of HTLV-I specific binding at 4°C and 25°C.** To establish the rate of specific binding of HTLV-I/R-16 to susceptible MT-2 cells and fairly resistant adult fresh PBMs, the parameters of temperature and time were varied (Fig 4). At 4°C, the assay measures viral adsorption alone, whereas at 25°C the virus can bind and penetrate the cells, possibly allowing for multiple sequential binding of a single cellular binding site. Fluorescent signals were recorded at minute intervals from the initial interaction of HTLV-I/R-16 and target cells. The rate of binding of HTLV-I/R-16 to MT-2 cells at 25°C proved very rapid and linear. No plateau was observed for 1-20 minutes. At 4°C, the rate of binding of HTLV-I/R-16 to MT-2 cells was much slower; within 15 minutes, 50% of the maximum cell-bound fluorescence was attained (Fig 4). The rate of binding subsequently plateaued at 60 minutes, followed by only minimal additional binding. These results are in accord with well established kinetics of specific virus–cell membrane receptor interactions at 4°C. In comparison, the maximum binding of HTLV-I/R-16 to fresh, nonactivated adult PBMs at 4°C and 25°C was much less. The adsorption plateau was reached at both temperatures more quickly than in MT-2 (Fig 4). As expected, the maximum bound PBM fluorescent was significantly higher at 25°C than at 4°C.

**Assay sensitivity.** The low limit of detectability of HTLV-I cell binding sites was determined by mixing a small proportion of known high HTLV-I binding site cells (MT-2) with a large proportion of cells with low HTLV-I cell binding activity (nonactivated PBMs). A mixture of 90% of PBMs and 10% MT-2 cells (Fig 5B) and another of 95% PBMs and 5% MT-2 cells (Fig 5C) showed 26% and 19% viable fluorescent cells above an intensity of 34 (the left edge of the MT-2 curve) whereas only 13% of the viable pure population of PBMs bound fluorescence above an intensity of 34 (Fig 5A). After subtraction of fluorescent-bound PBMs, the number of HTLV-I/R-16–bound MT-2 cells in these mixed populations were calculated as 13% and 16%, respectively. These results indicate that a subset of the PBMs was probably HTLV-I binding site positive and that as little as 5% and 10% HTLV-I cell membrane binding site-positive cells was accurately detected in these heterologous population of cells.

**Binding competition with an unrelated retrovirus.** To demonstrate further the specificity of this virus membrane binding assay, an ecotropic MLV, which has no significant
primary nucleotide sequence homology to HTLV-I and a restricted mouse or rat cell host range was used in cross-interference studies. Figure 6A shows histograms of MT-2 cells that revealed a twofold decrease in fluorescent intensity with HTLV-I homologous interference whereas only slight interference was visualized with MLV adsorption prior to HTLV-I challenge (Fig 6B). In contrast, MLV membrane binding to susceptible SC-1 cells and significant binding inhibition (fourfold) with homologous virus was clearly observed (Fig 6C).

Correlation between HTLV-I/R-16 adsorption and infectivity of target cells. Table I is a summary of HTLV-I/R-16 adsorption and attempted infection of human lymphoid target cells by cocultivation with HTLV-I producing donor cells. Table I is arranged in rank order of the highest to the lowest levels of HTLV-I/R-16 cellular adsorption. The data correlates directly with the transmission of HTLV-I to target cells by cocultivation with an equal number of irradiated, virus-positive T lymphoblasts. The MT-2, HUT 102-B2, MOLT 4, and normal adult-activated cycling PBMs bound HTLV-I/R-16 at mean fluorescence >10. The high relative mean fluorescence of HTLV-I/R-16 bound to HUT 102-B2 cells, is consistent with the high level of constitutive, endogenous infectious HTLV-I expression. Consequently, the amount of intracellular HTLV-I core antigen p19 detected by indirect immune fluorescent assay was equally elevated. Examination of chromosomal HTLV-I DNA by dot blot or Southern blot hybridization revealed intense dark dots or bands, indicative of multiple integrated copies of HTLV-I. In addition, virion-associated reverse transcriptase was detected with a specific template primer poly[rC]·oligo[dG] in the presence of Mg²⁺. MOLT 4, an immature T lymphoblast cell line, and normal activated adult mononuclear cells can be infected with HTLV-I, as indicated by results of similar virus transmission assays. The adsorption of HTLV-I/R-16 to HUT 78, FR45, and NK41, is approximately sixfold less than that to HUT 102-B2 cells. Homologous untagged HTLV-I failed to interfere significantly with HTLV-I/R-16 bindings in this former cell group. These data suggested relatively fewer binding sites and a lack of affinity for HTLV-I binding sites, which correlates well with the lack of HTLV-I transmission.

Two nonlymphoid, nonhuman fibroblast cell lines tested in the same experiment with the human lymphoid cell lines were African green monkey kidney epithelioid cells (VERO) and wild mouse fibroblasts (SC-1). Both these cell types are large in comparison to fresh mature lymphoid cells. In agreement with this size correlation, SC-1 and VERO cells both had large numbers of HTLV-I/R-16 binding sites with relative mean fluorescence of 1 20 and 59, respectively (data not shown), which was reduced approximately twofold with an equal concentration of competitive homologous unlabeled HTLV-I. At present, no evidence of HTLV-I infectivity has
been observed, but to prove conclusively a lack of HTLV-I transmission cocultivated target cells should be screened in a more sensitive assay such as the polymerase chain reaction coupled with Southern blot hybridization. The adsorption of virus to cell membranes does not always imply that the cell is permissive for infection. Susceptibility of cells to infection can be controlled at later points in the virus growth cycle beyond virus penetration into the cell.

**HTLV-I adsorption to normal human mononuclear targets.** Four normal donors were screened for HTLV-I/R-16 binding sites on their Ficoll-Hypaque density-gradient-purified mononuclear cells before and after activation of their T lymphocytes with PHA and growth in partially purified, delectinated IL 2 (Table 2). All nonactivated mononuclear target cells bound HTLV-I/R-16 to form a single peak of fluorescent cells. In contrast, the activated target cells were separated in two peaks of fluorescent cells based on intensity. The nonactivated PBMs were significantly more resistant to competition with unlabeled homologous HTLV-I, suggesting a lack of specificity. In three of four donors, one activated peak was significantly higher and the other lower than the nonactivated fluorescent cells (Table 2). In donor 4, the HTLV-I/R-16-bound cells with the highest intensity of fluorescence after activation were not significantly higher than the single peak of nonactivated target cells. These results were in accord with earlier studies showing that HTLV-I infection of normal mononuclear cells is more efficient in cycling, activated cells.* Analysis of T-enriched v non-T-enriched populations from these mononuclear preparations indicates that HTLV-I adsorption is greatest in the former population (Fig 3B).

**Competitive binding of HTLV-II.** Table 3 shows the results of cross-interference experiments between HTLV-I and HTLV-II in MT-2 and Mo-T cells initially adsorbed with one HTLV/R-16 and subsequently challenged with a homologous or heterologous HTLV/R-16. In MT-2 cells, HTLV-I/R-16 bound more effectively than HTLV-II/R-16, but the cross-interference patterns were very similar. HTLV-II blocked 66% of HTLV-I/R-16 binding, whereas HTLV-II inhibited 54% of its own adsorption. In Mo-T cells, constitutively producing HTLV-II, the number of HTLV-I binding sites was still higher than for HTLV-II. The homologous interference pattern of HTLV-II in Mo-T cells was slightly less than the homologous interference in MT-2 cells with HTLV-I.

**DISCUSSION**

In this report, a flow cytometric analysis used in previous Epstein-Barr virus (EBV) cell receptor studies is described for investigation of HTLV-I specific binding to fresh and cultured cells on a single cell basis. This assay used rhoda-

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### Table 1. Relationship Between HTLV-I Virus Adsorption and Infection in Human Lymphoid Cells

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>HTLV-I-Adsorption After Inhibition</th>
<th>Reverse Transcriptase Reaction</th>
<th>HTLV-I Infection</th>
<th>Immunofluorescence Cells Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HTLV-I Adsorption (Relative Mean Fluorescence)</td>
<td>With Unlabeled HTLV-I (Relative Mean Fluorescence)</td>
<td>Mixture</td>
<td>For HTLV-I p19 (%)</td>
</tr>
<tr>
<td>MT-2</td>
<td>35</td>
<td>15</td>
<td>10.50</td>
<td>+</td>
</tr>
<tr>
<td>HUT 102-B2</td>
<td>26</td>
<td>13</td>
<td>2.05</td>
<td>+</td>
</tr>
<tr>
<td>MOLT 4</td>
<td>17</td>
<td>10</td>
<td>1.79</td>
<td>+</td>
</tr>
<tr>
<td>PMB + *</td>
<td>13</td>
<td>8.0</td>
<td>0.85</td>
<td>+</td>
</tr>
<tr>
<td>HUT 78</td>
<td>8.2</td>
<td>8.0</td>
<td>0.12</td>
<td>-</td>
</tr>
<tr>
<td>FR-45</td>
<td>8.0</td>
<td>8.0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>NK-41</td>
<td>7.2</td>
<td>7.1</td>
<td>NT</td>
<td>-</td>
</tr>
</tbody>
</table>

NT, not tested.

*Activated normal adult peripheral blood mononuclear cells from normal donors.

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### Table 2. HTLV-I/R-16 Adsorption to Nonactivated and PHA-Activated Normal PMB Target Cells

<table>
<thead>
<tr>
<th>Donor PHA Fluorescence</th>
<th>HTLV-I/R-16 Cell Bound</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46</td>
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<tr>
<td></td>
<td>+</td>
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<tr>
<td>2</td>
<td></td>
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<tr>
<td>4</td>
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<td>+</td>
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</table>

*Both PHA-activated and nonactivated target cells were analyzed in the same experiment on the same day. In the PHA(−) samples, Ficoll-Hypaque density-gradient-purified mononuclear cells were immediately used in the above experiment. In the PHA(+) samples, 5 μg/mL PHA was added to target cell cultures for 24 hours. The cells were washed and resuspended in fresh media with 20% partially purified, delectinated IL 2. At 6 days, the virus adsorption assay was performed.

†Data represent relative mean fluorescence.
mine hydrocarbon-tagged, purified HTLV-I virions, according to the procedure originally published for avian retroviruses. Automated detection of HTLV-I binding by flow cytometry permitted rapid, sensitive, and reproducible testing. Accurate quantitation of virus binding to single cells could be performed on as few as 10^4 cells. The rate of virus-cell binding was temperature dependent, characteristic of all virus-cell receptor interactions. Only a specific virus-cell interaction was detected because nonrhodamine-tagged, homologous HTLV-I interfered with HTLV-I/R-16 binding significantly in susceptible but not resistant target cells. In contrast, unrelated, nonlabeled retroviruses were unable to block HTLV-I/R-16 binding in HTLV-I-sensitive target cells. Moreover, several target cell lines were insensitive to HTLV-I/R-16 adsorption.

The existing knowledge regarding the HTLV-I and HTLV-II cell membrane receptors is scarce as opposed to that which is known regarding the unrelated HIV. In contrast, the nature of the HIV-I cell receptor has been identified as an epitope of the CD4+ cell surface phenotype which is characterizedly found on normal helper T lymphocytes. The HTLV-I and HTLV-II receptors, which are known to differ from HIV-I, are still a mystery, although their distribution has been studied by us and other researchers. A summary of our studies in lymphoid and nonlymphoid cells follows. First, a direct relationship was shown between the expression of HTLV-I receptors on lymphoid targets and successful transmission of HTLV-I to the latter by cocultivation with irradiated, HTLV-I producing T lymphocytes. The target lymphoid cells are discussed in descending rank order of specific HTLV-I binding. As expected, HUT 102-B2 and MT-2, HTLV-I constitutively producing T lymphoblast cell lines expressed high levels of HTLV-I binding sites. This, in part, is a consequence of the relative large surface area of these cells. In reality, the number of binding sites may be much higher in these cells because some of the viral binding sites may be masked by previously bound, endogenous budding HTLV-I virions. These observations confirmed earlier studies using a fluoresceinated virus-cell binding assay analyzed on a flow cytometer. MOLT 4 is the only immature T cell line identified with HTLV-I binding sites and susceptible to infection. This neoplastic cell line was derived from a patient with acute lymphocytic leukemia. Four other established human T cell leukemia cell lines were studied by Sinangil et al. Two of four carried HTLV-I adsorption sites and were capable of HTLV-I infection. Weiss et al observed HTLV-I cell surface receptor on most T cell lines tested by an indirect VSV (HTLV) pseudotype infectivity assay. Enhancement of HTLV-I binding sites in a subpopulation of the mononuclear targets was noted with mitogen activation and IL 2 stimulation of fresh adult PBMs as compared with nonactivated control PBMs. This pattern was observed in three of four samples tested and in complement studies by Merl et al, who demonstrated that proliferating cycling T cells were more sensitive to HTLV-I transmission and subsequent transformation than were resting, nonstimulated T cells. Sinangil et al observed HTLV-I adsorption sites on fresh adult nonactivated T lymphocytes from a single donor by direct binding assays. All other lymphoid cells examined (including HUT 78, a non-HTLV-I-producing lymphoblast cell line from a cutaneous T cell lymphoma patient; FR45, a continuous normal, EBY-positive B cell line; and NK41, a continuous normal NK T cell line) were relatively resistant to HTLV-I adsorption. These results suggest that the latter target cells either have very low or no HTLV-I binding sites. Our results with the HUT 78 T cells confirm an indirect VSV (HTLV-I) pseudotype transmission analysis. Two other transformed B cell lines were examined previously. The two Burkitt's lymphoma B cell lines, Loukes and Raji, were highly to moderately positive for expression of HTLV-I binding sites without a direct correlation with HTLV-I transmission. Virus transmission may have proved successful if cocultivation with HTLV-I-producing T cells instead of cell-free virus infection had been used or if virus transmission had been examined at a much later time. An alternative explanation would be a postponement cellular restriction responsible for blockade of HTLV-I growth.

The relatedness of HTLV-I and HTLV-II envelope glycoproteins and their specific binding sites was also studied by us and by other researchers. Sodroski et al compared the primary structure of the exterior spike glycoprotein of HTLV-I and HTLV-II. The major glycoprotein of HTLV-I and HTLV-II showed 65% identity. Within the spike glycoprotein, similarity between the regions of hydrophilicity and hydrophobicity, glycosylation sites, and the location of cysteine residues suggested common secondary and tertiary structures. In addition, limited interference and cross-neutralization of VSV (HTLV-I) and VSV (HTLV-II) pseudotypes were observed, indicating that the envelopes of HTLV-I and HTLV-II are similar but antigenically distinct. Weiss and co-workers studied the relatedness of HTLV-I and HTLV-II envelope glycoprotein cell membrane receptor sites. Both viruses appear to recognize the same receptors, as shown by receptor interference of syncytium induction (cell fusion) and pseudotype infectivity, but HTLV-II induced cell synctia in a more restricted host cell range. Other differences also existed. Indeed, HTLV-I could be grown in a number of nonlymphoid cell types, whereas attempts to propagate HTLV-II in the same cells were unsuccessful.

The above findings were confirmed here by direct virus-cell binding analyses on the flow cytometer. HTLV-II was highly competitive with HTLV-I for MT-2–specific virus binding sites. HTLV-II bound fewer specific adsorption sites than did HTLV-I. This difference may reflect the difference in adsorption time of the nonlabeled and labeled virus and/or a lesser affinity of HTLV-II for the same receptor sites. Furthermore, there was more HTLV-I and HTLV-II specific adsorption of MT-2 cells than of Mo-T cells, neoplastic T lymphoblasts constitutively releasing HTLV-II. These observations correlate with the fact that MT-2 cells possess more integrated proviral HTLV copies and a faster growth rate than does Mo-T.

In two nonlymphoid, nonhuman cell lines, a direct correlation between HTLV-I adsorption sites and HTLV-I infection could not be established. The cell lines, African green monkey kidney cells (VERO) and wild mouse fibroblasts
(SC-I) bound high levels of HTLV-I virions, but viral transmission was not successful. These data directly indicate the presence of HTLV-I receptors. An indirect pseudotype infectivity assay and syncytia formation assay with T cells producing HTLV-I of 15 different nonlymphoid cells (including VERO) indirectly suggested the same conclusion. These studies suggested that HTLV-I receptors were present on all cells capable of VSV (HTLV-I) pseudotype propagation or cell fusion. Therefore, in nonhuman, nonlymphoid cell cultures, the cellular receptor restriction was not the major determinant of cell tropism of HTLV-I. In conclusion, our findings and further extended studies may lead to a clearer understanding of host susceptibility to disease, the pathogenesis of HTLV-I-induced ATL, and possible modification of host receptors for disease prevention.

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Specific adsorption of HTLV-I to various target human and animal cells

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