

## IN VITRO CELLULAR TROPISM OF HUMAN B-LYMPHOTROPIC VIRUS (HUMAN HERPESVIRUS-6)

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The human B-lymphotropic virus (HBLV),<sup>1</sup> recently isolated from the peripheral blood of six patients affected by various hematological disorders (1), was shown by detailed molecular and morphological characterizations to be a unique member of the *Herpesviridae* family (2, 3). HBLV is an enveloped virus, 200 nm in diameter, with an electron-dense icosahedral core composed of 162 capsomers (1, 3) containing a large double-stranded DNA genome (>110,000 bp). As a result of these observations, the taxonomic designation human herpesvirus-6 (HHV-6) was proposed (4, 5). Despite extensive seroepidemiological studies and attempts to obtain HBLV isolates from diversified patients, no definite etiological association with human disease has been established to date.

HBLV can infect fresh normal mononuclear cells in vitro, yielding characteristic morphologically altered lymphocytes, capable of surviving in culture for 15–20 d. Based primarily on the study of freshly isolated cells from infected patients, a B lymphocyte tropism was initially suggested for HBLV (1). However, subsequent in vitro studies demonstrated that HBLV could also infect cell lines of various origins, including B and T lymphocytes, megakaryocytes, and glial cells (4). In addition, two recent letters describing the isolation from African patients of human herpesviruses genetically indistinguishable from HBLV also suggested the infectability of other cell types (6, 7). Although in vitro studies using established cell lines are not necessarily predictive of the in vivo cellular tropism of a viral agent, the information so far available indicates that HBLV may have a wide range of infectable cells.

In the present report we investigated further the in vitro cellular tropism of HBLV using fresh normal mononuclear cells from various tissue sources. Infected cells were subjected to extensive immunological and molecular characterizations.

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<sup>1</sup> *Abbreviations used in this paper:* HBLV, human B-lymphotropic virus; MN, mononuclear cells; PE, phycoerythrin; TCID<sub>50</sub>, 50% tissue culture infectious dose; TdT, terminal deoxyribonucleotidyltransferase.

## Materials and Methods

*Source of Mononuclear Cells.* Heparinized samples of adult peripheral blood, bone marrow, and umbilical cord blood from healthy donors were collected under sterile conditions. Tonsils and thymuses, obtained from children undergoing cardiac surgery interventions, were extensively minced and the cells in suspension were washed out with sterile culture medium. Mononuclear cells (MN) from all sources were isolated by centrifugation on Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Piscataway, NJ).

*Source of Virus.* HBLV was propagated in fresh, activated umbilical cord blood leukocytes as previously described (1). For the in vitro infection of mononuclear cells, either conditioned medium from infected cord blood cultures or partially purified HBLV, obtained by density banding on sucrose gradients, was used. The titer or 50% tissue culture infectious dose (TCID<sub>50</sub>) was determined by infecting triplicate cultures of activated cord blood cells with serial 10-fold dilutions of the virus stock.

*Infection of Mononuclear Cells In Vitro.* Mononuclear cells, including the selected CD2<sup>-</sup> or CD3<sup>-</sup> subpopulations (see below), were initially activated by cultivation for 24 h at 37°C in humidified 5% CO<sub>2</sub> atmosphere in culture medium (RPMI 1640) supplemented with 15% FCS and 1 µg/ml PHA-P. Cells were then washed, pelleted, resuspended in 2 ml stock HBLV (titer ~10<sup>3</sup> TCID<sub>50</sub>) and incubated for 2 h at 37°C. The cells were subsequently washed and resuspended in culture medium supplemented with 10% FCS. 6–8 d after infection, dead cells were removed by Ficoll-Hypaque gradient centrifugation and infected cells were cultured for additional 2–4 d, before immunological or molecular characterizations. At that time, >90% of the cells were HBLV infected, as detected by IFA (see below) or by in situ hybridization with the specific probe pZVH14, as described (2).

*Immunofluorescence Analysis.* A wide panel of mouse mAbs and polyclonal antisera were used to characterize HBLV-infected cells by either direct or indirect IFA, as previously described (1, 8). Purified unlabeled, FITC or phycoerythrin (PE)-conjugated mAbs Leu-1, Leu-3a, Leu-4, Leu-7, Leu-9, Leu-11, Leu-M1, Leu-M2, Leu-M3, HPCA-1, anti-TCR-α/β heterodimer (WT31), anti-IL-2-R, anti-leukocyte antigen (HLe1) and anti-CR2 were purchased from Becton Dickinson & Co. (Mountain View, CA); OKT3, OKT4, OKT4a, OKT6, OKT8, OKT9, OKT11, OKDR, and OKBcALL from Ortho Pharmaceutical (Raritan, NJ); MY7, MY9, B1, and B4 from Coulter Immunology (Hialeah, FL); DRC and Kil from Dako Corp. (Santa Barbara, CA). Polyclonal rabbit anti-terminal deoxynucleotidyltransferase (TdT) was obtained from Bethesda Research Laboratories (Gaithersburg, MD); polyclonal goat anti-human IgG or IgM (γ or μ chain specific, respectively) from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Human sera containing specific antibodies towards HBLV were also used at 1:40 dilution for indirect IFA, after extensive absorption with HBLV<sup>-</sup> umbilical cord blood cells. Polyclonal FITC-conjugated goat anti-mouse IgG or IgM, goat anti-rabbit IgG and goat anti-human IgG antisera (Kirkegaard & Perry Laboratories, Inc.) were used as second antibodies. Cytoplasmic staining was carried out on cytocentrifuge preparations fixed for 10 min in cold acetone. Nuclear staining for TdT was performed on cytocentrifuge preparations fixed for 30 min in cold methanol. All samples were scored under a fluorescence microscope (Leitz), counting >200 cells per test. Flow cytometric analysis was performed using a Becton Dickinson & Co. FACS (FACS 440; Spectra Physics, Mountain View, CA) equipped with an Argon laser. Live infected cells were treated for 1 h with 0.5% paraformaldehyde before FACS analysis. Debris and dead cells were excluded from the analysis by conventional scatter setting system. Fluorescein (green) and PE (red) emission lights were collected with band pass filters 530/30 and 575/26, respectively, and logarithmically amplified. Isotype-matched mAbs of irrelevant specificity were used on the same cells as negative controls.

*Radio Immune Precipitation Assay (RIPA).* HBLV-infected cord blood cells and the control cell line ET62 were radioactively labeled by incubation for 18 h in medium containing 50 µCi/ml [<sup>35</sup>S]cysteine. Labeled cells were washed in PBS and disrupted with 10 nM sodium phosphate (pH 7.2) containing 0.5% NaCl, 1% Triton X-100, 0.5% sodium deoxycholate and 0.1% SDS (PBS-TDS). The lysate was adsorbed overnight at room tem-

perature with protein A-Sepharose. Immunoprecipitation analysis was performed by addition to labeled and clarified extract (1 ml) of 5  $\mu$ g purified OKT3 mAb and 0.2 ml of a 10% suspension of protein A-Sepharose bound to rabbit anti-mouse  $\kappa$  light chain. The samples were incubated overnight at 4°C. Immunoprecipitates were collected by centrifugation, washed repeatedly in PBS-TDS, resuspended in Laemmli sample buffer, heated for 2 min at 90°C, and analyzed by SDS-PAGE.

**Northern Blot Analysis.** Cellular RNA was extracted from HBLV-infected cord blood MN cells and control cell lines by the guanidine isothiocyanate procedure, as previously described (9). Total cellular RNA (20  $\mu$ g/lane) was electrophoresed through 1.2% formaldehyde-agarose gels and transferred to nylon membrane filters (Zeta Probe; Bio-Rad Laboratories, Cambridge, MA). After baking for 2 h at 80°C, filters were hybridized at 37°C for 12 h to nick-translated probes in 4 $\times$  SSC, 4 $\times$  Denhardt's solution, 0.1% SDS, 50% formamide, 10% dextran sulfate, 50 mM sodium phosphate pH 6.5, 250  $\mu$ g/ml yeast RNA. The filters were then washed two times in 2 $\times$  SSC/0.1% SDS at room temperature and two times in 0.2 $\times$  SSC/0.4% SDS at 65°C. The probes used for hybridization were labeled by nick translation to a specificity of  $\sim 10^9$  cpm/ $\mu$ g and included TCR $\beta$  (Bg1 II cDNA fragment derived from JUR- $\beta$ 2 clone, coding for most of the C $\beta$ 2 constant domain, a kind gift of Dr. T. Mak, Ontario Cancer Institute, Toronto Canada), TCR- $\alpha$  (cDNA clone pY14, a kind gift of Dr. T. Mak), TCR- $\gamma$  (derived from clone pT $\gamma$ -1, by subcloning into Eco RI sites of pGEM4, a kind gift of Dr. D. Dialynas, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA), and IgM heavy chain (a genomic subclone of 1.3 kb from the Eco RI site of C $\mu$  1 to the Eco RI site of C $\mu$  3 into the Eco RI site of pbr322, a kind gift of Dr. P. Leder, Dept. of Genetics, Harvard Medical School, Boston, MA).

**Immunoselection by Magnetic Microspheres.** Monosized magnetic microspheres coated with polyclonal goat anti-mouse IgG (DYNAL, Fort Lee, NJ) were used to isolate the CD2<sup>-</sup> or CD3<sup>-</sup> cell subsets, as described (10). Briefly, freshly isolated umbilical cord blood mononuclear cells ( $2 \times 10^7$ ) were incubated for 30 min at 4°C in PBS supplemented with 1% FCS with 2  $\mu$ g/ml of appropriate purified mAb. The cells were then washed three times with cold PBS and incubated at 4°C under continuous rotation in the presence of goat anti-mouse IgG-covered magnetic microspheres (40:1, beads/putative positive cells ratio). After 40 min the rosetting and nonrosetting cell populations were selected by placing the culture tube in contact with a cobalt-samarium magnet for 1 min and pipetting off the unbound cells. The proportion of residual CD2<sup>+</sup> or CD3<sup>+</sup> cells after this procedure was <1%, as detected by indirect immunofluorescence.

**Complement-mediated Cellular Cytotoxicity.** After treatment with magnetic beads, the depletion of CD2<sup>+</sup> or CD3<sup>+</sup> mononuclear cells was completed by complement-mediated cell lysis. CD2<sup>-</sup> or CD3<sup>-</sup> depleted subsets were incubated for 30 min at 4°C with the appropriate mAb (OKT11, OKT3, respectively, IgG2a subclass), washed three times, and resuspended in rabbit complement (PelFreeze Biologicals, Rogers, AR) for 1 h under continuous rotation. Cells were then washed three times in cold PBS and their viability was tested by Trypan blue dye exclusion.

## Results

**In Vitro Infection of Fresh Mononuclear Cells with HBLV.** Approximately 72 h after infection of PHA-treated cells, characteristic HBLV-infected cells began to appear within the cultures from all the tissue sources examined (Fig. 1). Little or no infection was observed when MN cells were exposed to the virus without previous in vitro activation by PHA. The morphologically altered infected cells subsequently became the predominant population, since the majority of the small, uninfected cells died within the first week in culture. The cell viability was restored to >90% after removal of dead cells at day 6–8 by Ficoll-Hypaque gradient centrifugation. After two more days in culture, >90% of the viable cells were HBLV<sup>+</sup>, as evaluated by indirect IFA or by in situ hybridization. All

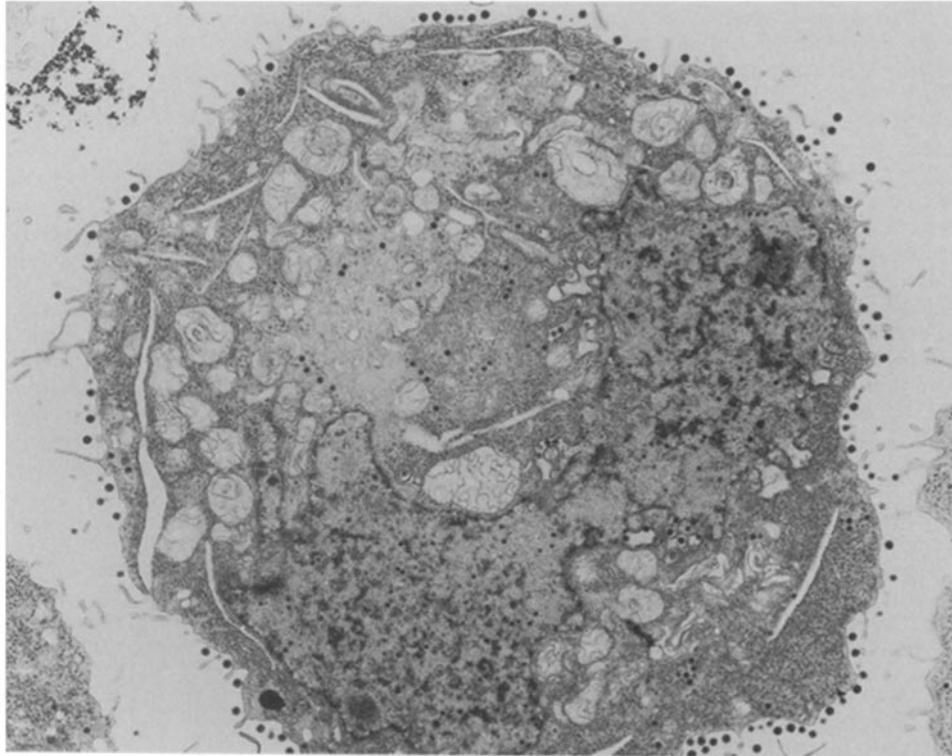


FIGURE 1. Characteristic electron microscopy appearance of an HBLV-infected T lymphocyte 8 d after infection. Mononuclear cells from normal human umbilical cord blood were infected with HBLV 24 h after activation with PHA. Intranuclear and intracytoplasmic immature particles are recognizable, as well as mature virions outside the cell.

the immunological and molecular tests were carried out on these homogeneous HBLV<sup>+</sup> cell populations. In the early phases of infection (day 5–8), the viable HBLV-infected cells demonstrated a consistent, albeit limited, spontaneous proliferative activity (compared with unstimulated MN cells from the same donors, as detected by [<sup>3</sup>H]thymidine incorporation, data not shown), but were unreactive to PHA and/or IL-2 (either crude or recombinant preparations) added to the cultures (data not shown).

*Immunological Analysis of HBLV-infected Cells.* As illustrated in Table I, HBLV-infected cells from normal adult peripheral blood, umbilical cord blood, or thymic tissues displayed phenotypic characteristics of immature T lymphocytes, by either direct or indirect IFA. Analogous results were obtained after infection of mononuclear cells from tonsil and bone marrow samples. The results depicted in the table refer to day 8–10 homogeneous HBLV-infected cell populations, but the same phenotypic pattern was also observed on single morphologically altered lymphocytes during the early phases of culture infection. Virtually all HBLV<sup>+</sup> cells expressed the CD7 antigen, the earliest known T-lymphocytic marker along the intrathymic T cell differentiation pathway, the CD5 antigen, a pan-T marker, and the CD2 antigen, the receptor for sheep erythro-

TABLE I  
*Immunological Characterization of HBLV In Vitro-infected Cells from Normal Human Peripheral Blood, Cord Blood, and Thymus*

Antigens	mAb	Adult PB	Umbilical CB	Thymus
CD1	OKT6	<1*	<1	<1
CD2	OKT-11	94.4	97.0	90.1
CD3	OKT3	<1 <sup>‡</sup>	<1 <sup>‡</sup>	<1 <sup>‡</sup>
CD4	Leu-3a	61.8	64.5	59.0
CD5	Leu-1	90.1	91.8	96.0
CD7	Leu-9	72.4	76.6	70.9
CD8	OKT8	26.7	15.7	25.3
CD15	Leu-M1	11.7	20.0	8.8
CD25	IL-2-R	<1	<1	<1
CD10, CD19, CD20, CD21, SIg, CIg <sup>§</sup>		<1	<1	<1
TCR	WT31	<1 <sup>‡</sup>	<1 <sup>‡</sup>	<1 <sup>‡</sup>
DR	OKDR	13.8	12.7	10.3
Leukocyte antigen	HLe1	95.5	97.4	91.6
Transferrin receptor	OKT9	91.8	95.3	90.1
Nuclear TdT <sup>  </sup>		<1	<1	<1
Others <sup>¶</sup>		<1	<1	<1

\* Mean value of percent IFA positivity from different samples (>3).

<sup>‡</sup> A low-intensity, diffuse cytoplasmic positivity was observed in the majority of the elements as detected on cytocentrifuge preparations fixed for 10 min in cold acetone.

<sup>§</sup> mAbs used: OKBcALL, B4, B1, CR2; polyclonal anti-IgG and anti-IgM ( $\gamma$  and  $\mu$  chain-specific, respectively).

<sup>||</sup> Cytocentrifuge preparations were fixed for 30 min in cold methyl alcohol.

<sup>¶</sup> Leu-M2, Leu-M3, OKM1, OKM5, MY7, MY9, DRC, Kil, Leu-7, Leu-11b, HPCA-1.

cytes, responsible for E-rosette formation. These cells also formed E-rosettes at 4°C in the presence of 25% FCS and 0.5% sheep erythrocytes (data not shown). Other antigens detected on the majority of the cells were CD4, transferrin receptor, and the leukocyte antigen HLe1, expressed at various intensities. A limited proportion of infected cells (15–25%) also expressed the CD8 antigen. Two-color immunofluorescence analysis showed that CD8<sup>+</sup> cells consistently coexpressed the CD4 antigen. The CD3 antigen, which appears on the cell surface during the last phase of the intrathymic T cell maturation, was not detected by IFA on the surface membrane but was weakly and diffusely expressed within the cytoplasm of infected cells. An mAb reported to bind to a nonpolymorphic region of the TCR- $\alpha/\beta$  heterodimer (WT31) (11), also yielded a weak cytoplasmic and no surface membrane signal. In addition to the above-mentioned antigens, a proportion of infected cells also coexpressed class II MHC DR antigens and the CD15 antigen (a granulocytic/monocytic marker also expressed by the Reed-Sternberg cells and some activated T cells, reference 12). HBLV-infected cells from all the sources studied did not display by IFA the early T cell marker CD1. In addition, nuclear TdT was not detected by a polyclonal rabbit antiserum on infected cells. Cells bearing B cell-associated antigens were only

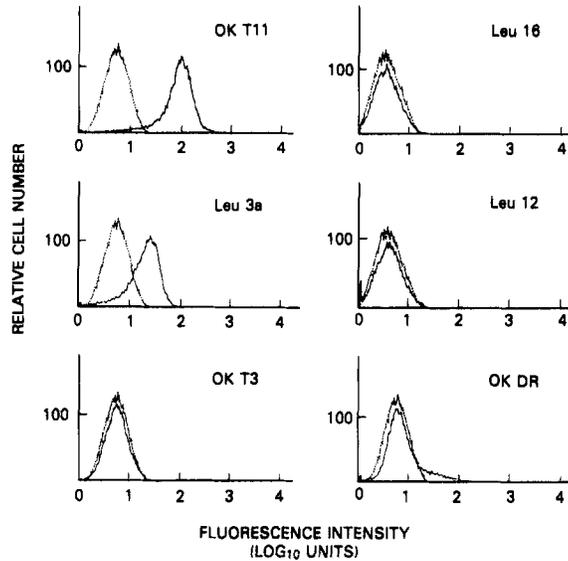


FIGURE 2. Flow cytometry analysis of HBLV-infected cells from normal human umbilical cord blood. HBLV-infected cell populations (> 90% HBLV<sup>+</sup> by indirect IFA with infected patient sera or by in situ hybridization with the RNA probe pZVH14) were washed three times in PBS and treated with FITC-conjugated (OKT11, OKT3, Leu-3a, OKDR) or PE-conjugated (Leu-16, Leu-12) mAbs. Cells were subsequently fixed with 0.5% paraformaldehyde and were analyzed. Dotted lines represent the profiles obtained with control, isotype-matched mAbs or irrelevant specificity, conjugated with the appropriate fluorochrome for each test antibody. Green and red emission lights were collected with band pass filters 530/30 and 575/26, respectively, and logarithmically amplified.

occasionally observed and consistently represented <1% of the infected population. A number of other antigens, characteristic of mononuclear phagocytic, dendritic, natural killer, myeloid precursor, or Reed-Sternberg cells, were not detected on infected cells. As shown in Fig. 2, fluorocytometric analysis of CD2, CD3, CD4, CD19, CD20, and DR antigens confirmed the results obtained by optical observation.

**Radioimmunoprecipitation (RIP) by Anti-CD3 mAb.** To confirm the IFA observation of low-level intracytoplasmic CD3 expression, an RIP assay was performed after metabolic labeling of HBLV-infected cells with [<sup>35</sup>S]cysteine. As shown in Fig. 3, the mAb OKT3 precipitated the CD3 light (Mol mass ~20 kD) and heavy (25 kD) subunits, together with the associated  $\beta$  and  $\alpha$  chains of TCR of ~43 and ~49 kD, respectively.

**Molecular Analysis of T and B Cell-specific Gene Expression.** To further confirm the T cell origin of HBLV-infected cells, the expression of specific mRNA for TCR- $\alpha$ , - $\beta$ , - $\gamma$  chains and Ig  $\mu$  heavy chains was studied. Fig. 4 depicts Northern blot analysis of total cellular RNA derived from HBLV-infected cord blood cells (lane A), the HTLV-I-infected T cell lines 702 and HUT-102 (lanes B and D,

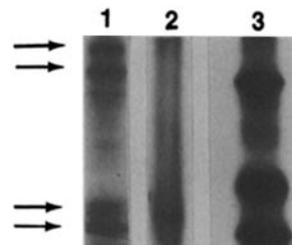


FIGURE 3. Radioimmunoprecipitation of [<sup>35</sup>S]cysteine-labeled human cord blood HBLV-infected cells and control EBV-transformed B-lymphoblastoid human cell line ET62. Cells were labeled overnight with 50  $\mu$ Ci/ml of [<sup>35</sup>S]cysteine and clarified lysates were immunoprecipitated with mAb OKT3. The precipitates were then subjected to SDS-PAGE analysis, under reducing conditions. (Lane 1) Cord Blood HBLV-infected cells; (lane 2) ET-62 control B cell line. The molecular weight standards (lane 3) include OVAL ( $42 \times 10^3$ ), carbonic anhydrase ( $31 \times 10^3$ ), soybean trypsinogen ( $21.5 \times 10^3$ ).

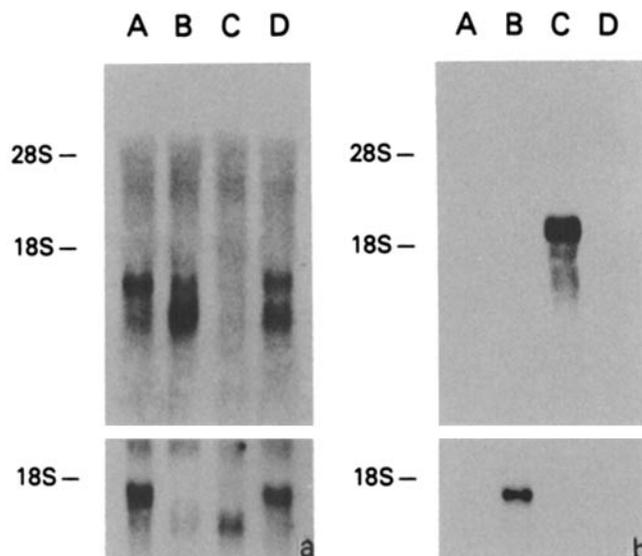


FIGURE 4. Northern blot analysis of TCR and IgM RNA expression in HBLV-infected cord blood cells. 20  $\mu$ g of total cellular RNA derived from HBLV-infected cord blood cells (a and b, lane A), HTLV-I-infected T cell lines 702 (lane B) and HUT-102 (lane D), and EBV-infected B-lymphoblastoid line RE (lane C) were electrophoresed under denaturing conditions and transferred onto nylon membrane filters. The filters were probed with cDNA fragment specific for TCR- $\beta$  (a, top panel), TCR- $\alpha$  (a, bottom panel), TCR- $\gamma$  (b, bottom panel), or a C $\mu$ -specific genomic clone (b, top panel). 18S and 28S ribosomal RNAs are indicated as size markers.

respectively), and the EBV-infected B cell line RE (lane C). Full-size TCR- $\alpha$  and - $\beta$  messages were identified in HBLV-infected cord blood cells (Fig. 4 a, top and bottom). TCR- $\gamma$  transcripts were not detectable in these cells (Fig 4 b, bottom), but were present in the control HTLV-I-infected T cell line 702. Similarly, mRNA for C $\mu$  was not identified in HBLV-infected cord blood cells, but was present in the control B cell line RE (Fig. 4 b, top). All lanes contained identical amounts of total RNA, as verified by ethidium bromide staining of the RNA gel.

**Infection of IL-2-dependent Cultured T Lymphocytes.** In addition to fresh normal mononuclear cells, normal T lymphocytes expanded *in vitro* in the presence of IL-2 for >1 mo were successfully infected with HBLV (Fig. 5). The yield of infected cells from IL-2-dependent cultures was definitely lower than using fresh mononuclear cells and progressively declined in long-term IL-2-stimulated cultures. Very limited infection was indeed observed exposing the HBLV IL-2-dependent T lymphocytes cultured *in vitro* for >60 d.

**Infection of Selected Mononuclear Cell Subsets.** To further address the question of the origin and maturation level of HBLV target cells, CD2<sup>-</sup> or CD3<sup>-</sup> MN cell populations were obtained from human cord blood by means of immunoselection with mAbs and goat anti-mouse IgG-coated magnetic microspheres, followed by complement-mediated cell lysis. Control unfractionated MN cells were exposed to rabbit complement alone, without previous mAb treatment. Equal numbers of selected and unselected cells were subsequently activated with PHA, infected with HBLV, and placed into culture, as described above. As illustrated in Fig. 6, the absolute number of HBLV-infected cells recovered from the CD3<sup>-</sup> MN cell populations of three different donors 10 d after infection was comparable to that from total MN cells (mean  $\pm$  SD from  $10^7$  initial cells:  $2.3 \pm 0.3 \times 10^6$  from total MN cells vs.  $2.1 \pm 0.7 \times 10^6$  from CD3-depleted fractions). By contrast, virtually no infected cells were detectable after 10 d in CD2-depleted cell cultures (mean  $0.1 \pm 0.1 \times 10^6$  from  $10^7$  initial cells).

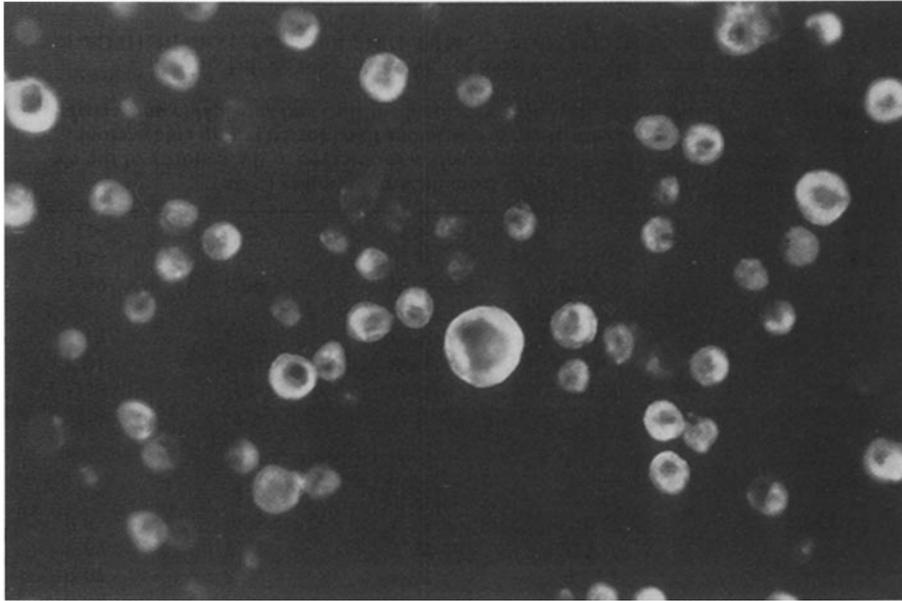


FIGURE 5. Indirect immunofluorescence analysis of normal human peripheral blood T lymphocytes exposed to HBLV 40 d after establishment in culture. At day 1 the cells were stimulated with PHA-P (1  $\mu\text{g}/\text{ml}$ ) and since day 4, delectinated IL-2 was regularly added at 10% final dilution. Cyto centrifuge preparations were fixed in cold acetone for 10 min. Serum from an HBLV<sup>+</sup> human subject was used at 1:40 dilution. FITC-conjugated polyclonal anti-human IgG ( $\gamma$  chain specific) antiserum was used as second layer antibody.

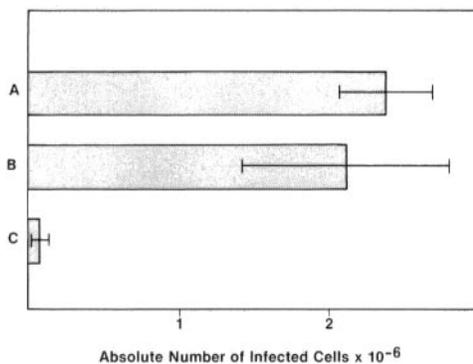


FIGURE 6. Infection by HBLV of unselected and selected mononuclear cell populations from human cord blood. The number of infected cells was evaluated at day 10 after exposure to the virus of  $10^7$  fresh total (A), CD3-depleted (B), or CD2-depleted (C) mononuclear cells. The percentage of infected cells in the cultured population was established by indirect immunofluorescence analysis with positive human sera. The values are absolute counts of infected cells and represent the mean  $\pm$  SD from three different cord blood donors. The selection procedures by magnetic microspheres followed by complement-mediated cell lysis are given in Materials and Methods.

### Discussion

HBLV was originally isolated from the peripheral blood of six patients affected by diverse apparently unrelated, hematological disorders (1). A common feature of these patients was, however, a condition of immune deficiency, either of iatrogenic origin (due to chemotherapeutic regimens) or caused by their own primary diseases (two, for example, were HIV-1 infected). This abatement of the immune defenses could account for the susceptibility to infection by HBLV or for the activation of latent virus. Recent seroepidemiological studies

by means of immunofluorescence techniques on fixed HBLV-infected cells showed a widespread high prevalence of HBLV-specific antibodies in several hematological disorders, of both neoplastic and non-neoplastic origin (Ablashi, D. V., et al., manuscript in preparation; Streicher, H. Z., et al., manuscript in preparation). In particular, significant seropositivity was observed in various lymphoproliferative disorders, advanced acquired immune deficiency syndrome (AIDS), and the so-called chronic mononucleosis syndrome or chronic fatigue syndrome (13). In addition, HBLV-specific DNA was recently identified by Southern blot analysis within the neoplastic tissues from three patients affected by non-Hodgkin's lymphoma of B cell origin (14). Despite these observations, no definitive evidence to date has linked HBLV to any specific clinical entity in humans.

The present report demonstrates that HBLV is electively infectious for fresh T lymphocytes *in vitro* and exerts on them a strong cytopathic effect. Despite considerable efforts to elucidate the maturation stage of the T cells susceptible to HBLV infection, it remains questionable whether they belong to a discrete, quasimature differentiation phase or, conversely, are fully mature lymphocytes undergoing important phenotypic and functional alterations after the interaction with the virus. In this study, HBLV-infected T cells displayed some immature phenotypic characteristics (15). For example, they lack the surface membrane TCR/CD3 complex and coexpress, at least partially, the CD4 and CD8 antigens. The simultaneous expression of these latter markers is characteristic of the cortical thymic population, which is largely CD1<sup>+</sup> (16). The lack of CD1 determinants on HBLV-infected cells (included those derived from thymic tissues) is not indicative *per se*, since it has recently been reported (17) that CD1 expression is not an indispensable intermediate step along the T cell differentiation pathway outside the thymic environment.

In contrast with the immature phenotypic features, however, the analysis at the RNA level demonstrated a mature pattern of TCR gene expression by HBLV-infected cells. Indeed, HBLV-infected cells displayed full-size TCR- $\alpha$  and - $\beta$  chain, but not - $\gamma$  chain mRNA, which is expressed at early stages of T cell maturation (18). In addition, the nuclear TdT, another early T cell marker, was negative on HBLV-infected cells. We attempted to address the question of the maturation level of the HBLV target cells by infecting immunoselected mononuclear cell populations. The results of these experiments indicate that at least a consistent proportion of the susceptible cells resides within the CD3<sup>-</sup>, CD2<sup>+</sup> immature T cell compartment. Another indication of immaturity is provided by preliminary data from our laboratory, suggesting that HBLV-infected T cells are unable to secrete the lymphokine IL-2 after stimulation *in vitro* with PHA and phorbol esters.

As further evidence of the T cell tropism of HBLV, we also showed that normal T lymphocytes maintained in culture in the presence of IL-2 for >1 mo were easily infected. Furthermore, it was previously reported that several established cell lines of various lineage origin, including T cells at early stages of differentiation, could be infected by HBLV (19). In contrast, it is possibly relevant that the T cell lines H9 and PEER, which are apparently refractory to HBLV infection (Lusso, P., preliminary data), display a phenotype corresponding to an advanced T cell maturation stage (surface CD3<sup>+</sup>).

While it is clear from this study that T cells represent a major target for HBLV infection, a linkage with B cell-related lymphoproliferative disorders is suggested by the direct detection of HBLV in tumor tissues from non-Hodgkin lymphomas of B cell type (14). This may suggest an indirect role for B cell tumorigenesis as was hypothesized for HIV-1 (20, 21) or HTLV-I (22), presumably acting through chronic antigenic stimulation and suppression of the T cell surveillance on immune-activated or EBV-transformed B lymphocytic clones. In light of the cellular tropism manifested by HBLV in vitro, we are currently investigating the possible involvement of this virus in the pathogenesis of various T cell-related hematological disorders, as well as of neoplasms of B lymphocytic origin. In addition, due to the dramatic cytopathic effect exerted in vitro on T lymphocytes, we are considering a possible role of HBLV in immunodeficiency conditions.

### Summary

We investigated the cellular tropism of human B-lymphotropic virus (HBLV) (also designated Human Herpesvirus-6) in vitro by infecting fresh MN cells from normal human adult peripheral blood, umbilical cord blood, bone marrow, tonsil, and thymus. Cultures from all the sources examined contained infectable cells, as shown by the appearance of characteristic enlarged, round-shaped, short-lived cells expressing HBLV-specific markers. Detailed immunological analysis demonstrated that the vast majority of these cells expressed T cell-associated antigens (i.e., CD7, CD5, CD2, CD4, and to a lesser extent, CD8). The CD3 antigen and the TCR- $\alpha/\beta$  heterodimer were not detectable on the surface membrane, but were identified within the cytoplasm of HBLV-infected cells, by both immunofluorescence and radioimmunoprecipitation assay. A proportion of the HBLV-infected cell population also expressed the CD15 and class II MHC DR antigens. By means of immunoselection procedures it was possible to show that a consistent proportion of HBLV-infectable cells were contained within the CD3-depleted immature T cell population, while the depletion of CD2<sup>+</sup> cells completely abrogated the infectability of the cultures. Northern blot analysis confirmed the T cell origin of HBLV-infected cells, demonstrating the expression of full size TCR- $\alpha$  and - $\beta$  chain mRNA. In addition to fresh T cells, HBLV was able to infect normal T lymphocytes expanded in vitro with IL-2 for >30 d.

These results indicate that HBLV is selectively T cell tropic in the course of the in vitro infection of normal mononuclear cells and may therefore be directly involved in the pathogenesis of T cell related hematological disorders. In particular, in light of the cytopathic effect exerted in vitro on CD4<sup>+</sup> T lymphocytes, a possible role of HBLV in immune deficiency conditions should be considered.

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