

HUMAN T CELL LEUKEMIA/LYMPHOMA VIRUS I
INFECTION AND SUBSEQUENT CLONING OF NORMAL
HUMAN B CELLS

Direct Responsiveness of Cloned Cells to Recombinant Interleukin 2 by
Differentiation in the Absence of Enhanced Proliferation

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Human T cell leukemia/lymphoma virus (HTLV)-I can infect and transform normal T cells (1), and has also been found in some transformed B cell lines (2, 3). HTLV-I infection is associated with a profound increase in the number of cell surface interleukin 2 (IL-2) receptors (containing the Tac antigen) on infected T cells (1, 4). The ability of B lymphocytes to also express IL-2 receptors under certain conditions (2, 3, 5-7), and the precise manner by which IL-2 affects B cell function, are areas of intense current interest (3, 5-8). Since we are interested in examining the effects of IL-2 on a homogeneous population of human B cells, we attempted to establish HTLV-I-infected, IL-2 receptor-positive B cell lines from normal human B cells. We show that HTLV-I can infect normal mature human B cells in vitro, and that IL-2 can affect these B cells, through their IL-2 receptor, to directly induce the differentiation of HTLV-I-infected B cells into Ig-secreting cells, without affecting their proliferation.

Materials and Methods

B Cell Purification. Human tonsils were processed to obtain purified resting B cells using counterflow centrifugation elutriation (Model J-6B; Beckman Instruments, Inc., Palo Alto, CA) as previously described (6). The smallest-sized B cell fractions (mean volume of 160-180 μm^3) were >95% surface Ig-positive and <0.2% T3⁺, as determined by fluorescence-activated cell sorter (FACS) analysis.

HTLV-I Infection. Cocultivation of the HTLV-I⁺ B cell line, HS1, a B cell clone isolated from a female patient with HTLV-associated adult T cell leukemia, with highly purified tonsillar small resting B cells was carried out as previously described (2). After 2 wk in culture, cell lines grew spontaneously and displayed growth in large clumps. Cell lines obtained from coculture of HS1 and unactivated B cells were called B-HTLV. After several weeks in culture, the cell line was examined for HLA phenotype and determination of HTLV infection. Cells infected with HTLV-I were identified by indirect immunofluorescence using a monoclonal antibody (mAb) against p19, an HTLV-I-encoded protein in the cytoplasm of the infected cell, and by Southern blot hybridization using an HTLV-I *pol-env-pX* probe. Clonal derivatives were obtained from B-HTLV by limiting dilution,

with cells seeded at mean concentrations of 10, 5, 1, and 0.3 cells/well. A positive well was selected for further characterization from plates seeded at the lowest cell concentration (0.3 cells/well) where only 3 of 120 wells were positive. This line was designated STB-1. These cell lines and clones have thus far been maintained for 4 mo in culture.

Southern Blotting Study. Southern blot hybridization was processed using a DNA probe, a ^{32}P -nick-translated DNA insert from a subclone in pBR322 of the portions of the HTLV-I genome defined by the Cla I and Hind III sites of $\lambda\text{CR-1}$ and containing *pol-env-pX* sequences (9).

Cell Staining. The mAb used to stain cells for FACS analysis were: anti-Tac in ascites form, generously provided by Dr. Thomas Waldmann (NIH); control ascites (P3X63); OKT3 (Ortho Diagnostic Systems, Inc., Raritan, NJ); and Leu-1 or Leu-12 (Becton Dickinson Monoclonal Center, Inc., Mountain View, CA). Surface Ig staining was done directly with fluorescent goat anti-human Ig (Cappel Laboratories, Cochranville, PA).

Factors. Concanavalin A (Con A)-stimulated supernatant was prepared by coculturing peripheral blood mononuclear cells of two allogeneic donors in the presence of Con A (10 $\mu\text{g/ml}$). Purified IL-1 (Genzyme Corp., Boston, MA), recombinant IL-2 (Lot LP210; provided by Cetus Corp., Emeryville, CA), and recombinant γ -interferon (IFN- γ) (Genentech Corp., So. San Francisco, CA) were used in this study. B cell growth factor (BCGF) was derived from a B cell line, Namalva, as previously described (10).

Culture Conditions and Assays. HTLV-I-infected B cell lines or clones were cultured in 0.2 ml of RPMI-1640 supplemented with 10% fetal calf serum, and gentamicin (20 $\mu\text{g/ml}$) in 96-well, flat-bottomed microtiter plates (3072; Falcon Labware, Oxnard, CA) at a cell density of 5×10^3 cells/well. Assays for proliferation and differentiation of cultured cells were done as previously described (6).

Results and Discussion

Development of HTLV-I-infected B Cell Clones In Vitro. The cell surface characteristics of the cell lines used are listed in Table I. The newly established cell lines could be distinguished from HS1 by differences in sex chromosomes and HLA antigens, HS1 was isolated from a female patient, and expressed HLA-DR3, MT1, and MT2. The new cell lines (B-HTLV, and STB-1) contained a Y chromosome, and expressed HLA-DR6, DR10, MT1, and MT2. These cell lines expressed B cell-associated antigens, surface Ig and Leu-12, but neither of the T cell markers, Leu-1 nor T3. They also expressed the HTLV-encoded viral protein, p19, in their cytoplasm. In addition, we determined whether STB-1 cells were infected with HTLV-I by Southern blot hybridization (Fig. 1). When DNA from the cells were digested with Bam HI and hybridized with HTLV-I *pol-env-pX* probe, three distinct bands and three thin bands, which contained

TABLE I
Characterization of HTLV-I-infected Cells

Marker	Normal B cells	HS1*	B-HTLV [‡]	STB-1 [§]
Surface Ig	+	+	+	+
Leu-12	+	+	+	+
Leu-1	-	-	-	-
OKT3	-	-	-	-
p19	-	+	+	+
Tac	-	+	+	+
HLA-DR	+	+	+	+

* HS1 is an HTLV-I-transformed B cell clone isolated from a patient with HTLV-I-associated adult T cell leukemia.

[‡] B-HTLV is an HTLV-I-infected B cell line obtained by coculture of HS1 with unactivated B cells.

[§] STB-1 is a B cell clone obtained from the B-HTLV cell line by limiting dilution.

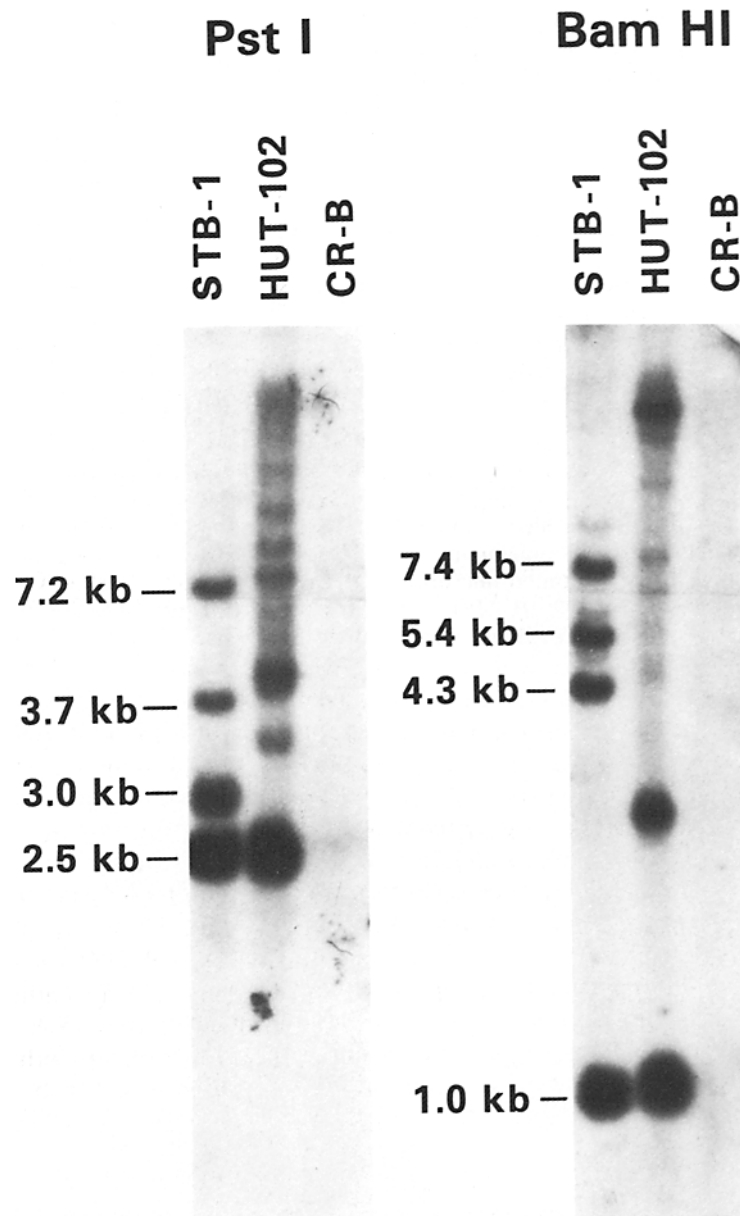


FIGURE 1. Proviral sequences of HTLV-I in clone STB-1. DNA digested with Pst I and Bam HI was analyzed by Southern blot. DNA was obtained from STB-1, an HTLV-I_{CR}-bearing tumor cell line, HUT-102, and an EBV-transformed, HTLV-I-uninfected B cell line, CR-B. Proviral bands are indicated, and sizes, in kb, are shown.

Bam HI junction fragments, were obtained, indicating that three copies of HTLV-I were integrated in this population. A distinct band of 1.1 kilobases (kb) was also observed, which is common to all HTLV-I-infected cells, and represents an internal fragment of the provirus. This indicates that there is not a large internal deletion of the proviruses in STB-1 cells. Digestion with Pst I resulted in the internal fragment of 2.5 kb and three junction fragments, confirming that

TABLE II
Responses of HTLV-I-infected B Cells to Various Lymphokines

Cells	Lymphokine	Dose	[³ H]Thymidine	IgM production*
			uptake*	
			<i>cpm</i>	<i>ng/ml</i>
HS1	Background	—	1,144 ± 189	1,138 ± 60
	Con A supernatant [‡]	10% vol/vol	2,579 ± 102	4,345 ± 771
	IL-2	25 U/ml	1,679 ± 80	1,530 ± 80
B-HTLV	Background	—	1,432 ± 171	171 ± 31
	Con A supernatant	10% vol/vol	1,077 ± 14	728 ± 100
	IL-2	25 U/ml	1,153 ± 85	1,301 ± 102
STB-1	Background	—	1,162 ± 81	193 ± 15
	Con A supernatant	10% vol/vol	1,489 ± 153	520 ± 35
	IL-2	25 U/ml	1,274 ± 25	1,279 ± 48
	IL-1	5 U/ml	1,265 ± 336	137 ± 13
	IFN- γ	200 U/ml	1,256 ± 130	178 ± 14
	BCGF-Namalva	10% vol/vol	1,412 ± 109	180 ± 18

* For details of assays, see Materials and Methods. Data represent mean \pm SEM of triplicate cultures.

[‡] Con A supernatant contained 8 U/ml of IL-2.

three oligoclonal proviral integrations took place in this population. The new cell lines were also tested for Epstein-Barr virus (EBV) infection, as determined by the presence of Epstein-Barr nuclear antigen (EBNA). Some cells of each cell line expressed a small amount of EBNA. However, at the time we infected the small B cells with HTLV-I, EBNA was undetectable. Thus, they still were "normal" B cells at the time they became transformed with HTLV-I.

Nonactivated small resting B cells are negative for Tac antigen, whereas in vitro-activated B cells do express Tac antigen on their surface (3, 5–7). As shown in Table I, expression of Tac antigen on the cell surface of normal resting B cells was induced by in vitro infection with HTLV. Several investigators have demonstrated a strong correlation between the expression of Tac antigen on T cells and infection with HTLV-I (1, 4). This suggests that HTLV may either upregulate the expression of Tac antigen on infected cells, or may itself produce a protein, similar or identical to the Tac antigen, that becomes expressed on the cell surface, but there is little evidence for HTLV-I encoding a Tac-like antigen (11). Nevertheless, the possibility that HTLV-I selectively infected a small number of already Tac⁺ cells cannot be ruled out by this study.

Responses of HTLV-I-infected B Cells to Various Lymphokines. To determine the effect of various lymphokines on HTLV-I-infected B cells, cells were incubated at 5×10^3 cells/well with a variety of lymphokines, after which DNA synthesis and Ig production were measured (Table II). HS1 cells spontaneously produced IgM, but not IgG or IgA, in the absence of lymphokines. This cell line could be induced to produce additional IgM by Con A supernatant (containing a variety of lymphokines, including IL-2 and B cell differentiation factor). Low concentrations (25 U/ml) of IL-2 had little effect on the proliferation and differentiation of HS1. The new lines, B-HTLV and STB-1, had a relatively low background secretion of IgM, and virtually no background secretion of IgG or IgA. However, B-HTLV responded well to IL-2 with an eightfold enhanced secretion of IgM. The same was true of STB-1, which secreted IgM in response to IL-2 to a greater degree than to the other lymphokines tested. IL-2 did not enhance the proliferation of these B cells at any concentration tested (0.8–100 U/ml). Fig. 2 A shows

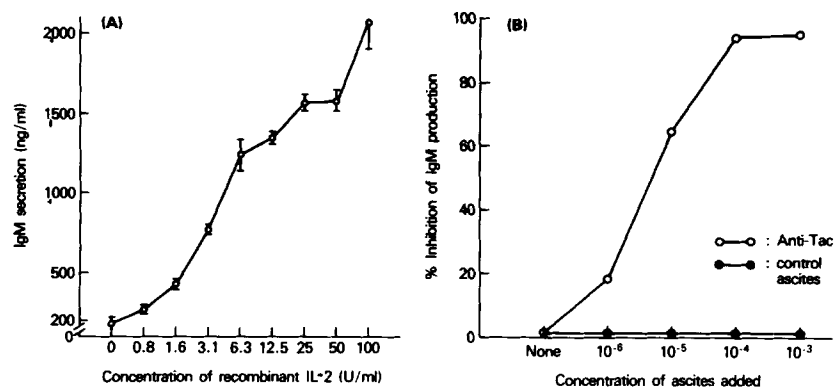


FIGURE 2. (A) Concentration-dependent effect of IL-2 on the differentiation of an HTLV-I-infected B cell clone, STB-1. Data represent the mean \pm SEM of triplicate cultures. (B) Effect of anti-Tac antibody on the IL-2 induced differentiation of an HTLV-I-infected B cell clone, STB-1. STB-1 cells cultured with 25 U/ml of recombinant IL-2 in the presence of anti-Tac ascites (○—○) or control ascites P3X63 (●—●). Data are expressed as percent inhibition of the response obtained in cultures receiving medium or recombinant IL-2 alone (195 ± 15 ng/ml and $1,570 \pm 29$ ng/ml), respectively.

the concentration-dependent effect of recombinant IL-2 on the differentiation of STB-1, as measured by the secretion of IgM. Neither highly purified IL-1, recombinant IFN- γ , nor BCGF produced by the B cell line Namalva (10) modulated DNA synthesis or IgM production by the clone STB-1 at any concentration tested. Clearly, IL-2 alone can induce substantial IgM production in this system.

The IL-2-induced differentiation of STB-1 cells was notably inhibited, in a concentration-dependent manner, by the addition to culture of anti-Tac antibody, but not by control ascites (P3X63) (Fig. 2 B). In addition, anti-Tac antibody had no effect on the background proliferation of STB-1 cells (data not shown). These data suggest that Tac antigen found on the HTLV-I-infected B cell clone STB-1 has functional importance in the IL-2-induced differentiation of this clone.

The interaction between IL-2 and its receptor on B cells is of interest. Ralph et al. (8) shows that Tac antigen was not involved in the IL-2-induced differentiation of EBV-transformed B cells. However, as Waldmann et al. (3) have shown, EBV-transformed B cell lines derived from Tac⁺, activated normal B cells continued to express Tac antigen, and were induced to differentiate by the addition of IL-2. In addition, Tsudo et al. (5) suggested that IL-2 induced the proliferation of normal activated B cells via the IL-2 receptor, as defined by the Tac antigen. These observations, taken in conjunction with this study, suggest that the IL-2 receptor is induced in certain subsets of B cells, or B cells at certain stages of differentiation, and that IL-2 can perform an important role in B cell maturation through its receptor. Furthermore, HTLV-I, different from EBV, may induce B cells to respond to IL-2 by inducing the expression of the IL-2 receptor. However, it is still not clear whether the IL-2 receptor is somehow closely associated with a differentiation receptor, or whether IL-2, acting through its own receptor on the responding cell, provides a second late differentiation or secretion signal.

Summary

A human T cell leukemia/lymphoma virus (HTLV)-I-infected B cell clone expressed Tac antigen on its cell surface and responded to recombinant interleukin 2 (IL-2) by increased production of IgM without any increase in proliferation. Anti-Tac antibody completely inhibited the IL-2-induced differentiation of this HTLV-I-infected B cell clone. This study demonstrates that HTLV-I can directly infect normal mature human B cells, and that the Tac antigen, which may be induced by infection with HTLV-I, is the functional receptor for IL-2-induced B cell differentiation. The availability of such cell lines and clones should provide useful tools to delineate precisely the differentiation step in the human B cell cycle.

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References

1. Broder, S., P. A. Bunn, E. S. Jaffe, W. Blattner, R. C. Gallo, F. Wong-Staal, T. A. Waldmann, and V. T. DeVita. 1984. T-cell lymphoproliferative syndrome associated with human T-cell leukemia/lymphoma virus. *Ann. Intern. Med.* 100:543.
2. Longo, D. L., E. P. Gelmann, J. Cossman, R. A. Young, R. C. Gallo, S. J. O'Brien, and L. A. Matis. 1984. Isolation of HTLV-transformed B lymphocyte clone from a patient with HTLV-associated adult T cell leukemia. *Nature (Lond.)* 310:505.
3. Waldmann, T. A., C. K. Goldman, R. J. Robb, J. M. Depper, W. J. Leonard, S. O. Sharrow, K. F. Bongiovanni, S. J. Korsmeyer, and W. C. Greene. 1984. Expression of interleukin 2 receptors on activated human B cells. *J. Exp. Med.* 160:1450.
4. Lando, Z., P. Sarin, M. Megson, W. C. Greene, T. A. Waldmann, R. C. Gallo, and S. Broder. 1983. Association of human T-cell leukaemia/lymphoma virus with the Tac antigen marker for the human T-cell growth factor receptor. *Nature (Lond.)* 305:733.
5. Tsudo, M., T. Uchiyama, and H. Uchino. 1984. Expression of Tac antigen on activated normal human B cells. *J. Exp. Med.* 160:612.
6. Muraguchi, A., J. H. Kehrl, D. L. Longo, D. J. Volkman, K. A. Smith, and A. S. Fauci. 1985. Interleukin 2 receptors on human B cells. Implications for the role of interleukin 2 in human B cell function. *J. Exp. Med.* 161:181.
7. Nakagawa, T., T. Hirano, N. Nakagawa, K. Yoshizaki, and T. Kishimoto. 1985. Effect of recombinant IL-2 and γ -IFN on proliferation and differentiation of human B cells. *J. Immunol.* 134:959.
8. Ralph, P., G. Jeong, K. Welte, R. Mertelsmann, H. Rabin, L. E. Henderson, L. M. Souza, T. C. Boone, and R. J. Robb. 1984. Stimulation of immunoglobulin secretion in human B lymphocytes as a direct effect of high concentrations of IL-2. *J. Immunol.* 133:2442.
9. Manzari, V., F. Wong-Staal, G. Franchini, S. Colombini, E. P. Gelmann, S. Oroszlan, S. Staal, and R. C. Gallo, 1983. Human T-cell leukemia-lymphoma (HTLV): cloning of integrated defective provirus and flanking cellular sequences. *Proc. Natl. Acad. Sci. USA.* 80:1574.
10. Ambrus, J. L., and A. S. Fauci. 1985. Human B lymphoma cell line producing B cell growth factor. *J. Clin. Invest.* 75:732.
11. Leonard, W. J., J. M. Depper, G. R. Crabtree, S. Rudikoff, J. Pumphrey, R. J. Robb, M. Krönke, P. B. Svetlik, N. J. Peffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. *Nature (Lond.)* 311:626.