# Rapid Communication

## Expression of p55 (Tac) Interleukin-2 Receptor (IL-2R), But Not p75 IL-2R, in Cultured H-RS Cells and H-RS Cells in Tissues

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*The authors studied the secretion of interleukin-2 (IL-2), the expression of interleukin-2 receptors (IL-2R; p55/Tac and p75), and the response to exogenous IL-2 by cultured Hodgkin's Reed–Sternberg cells (cell lines HDLM-1, HDLM-1d, and KM-H2) and T cells (H9, HuT78, HuT102, MOLT-4, and MT-2). All of these cells did not produce IL-2 or produced it in undetectable amounts, and their growth was not affected by the addition of anti-IL-2 or anti-IL-2R antibodies. This indicates that H-RS cells in long-term culture, as well as T cells, can grow independently of IL-2. The three H-RS cell lines, as well as two of the T-cell lines (HuT102 and MT-2), expressed Tac, whereas the other three T-cell lines were Tac negative. Expression of p75 was noted in the two Tac-positive T-cell lines, but not in cultured H-RS cells. The expression of Tac and p75 in HuT102 and MT-2 cells correlated well with their capacity to proliferate on treatment with exogenous IL-2. On IL-2 treatment, nucleic-acid uptake in Tac/p75-positive T cells increased approximately four- to sixfold, whereas the Tac/p75-negative T cells did not show increased proliferation. Unlike the T cells, the Tac-positive H-RS cells did not respond to IL-2. The lack of a proliferative response to IL-2 appears to be related to the absence of p75 in H-RS cells. A similar pattern (Tac positivity and p75 negativity) was noted in H-RS cells in lymph nodes involved by Hodgkin's disease. Thus the exogenous IL-2 released by surrounding T lymphocytes may not cause the proliferative activity of H-RS cells because of the lack of high-affinity IL-2 receptors in the latter cells. In contrast to H-RS cells in culture, H-RS cells in tissues were stained by a specific anti-IL-2 monoclonal antibody. This indicates that the expression of IL-2 or an IL-2-like substance by H-RS cells in tissues may be responsible, in part, for the great increase in the number of reactive T lymphocytes in tissues involved by Hodgkin's disease. Am J Pathol 1990, 136:735–744)*

Interleukin 2 (IL-2) is a polypeptide growth factor that stimulates the proliferation and function of T cells, B cells, natural killer cells, and other cytotoxic effector cells after it binds to specific receptors.[1,2] Most IL-2 receptors (IL-2R) have a low affinity (kd $\cong$ 10 nmol) for IL-2 and consist of a 55-kda polypeptide chain (Tac antigen, p55). High-affinity receptors (Kd $\cong$ 0.01 to 0.1 nmol) represent, in most cases, about 10% of the IL-2R and are implicated in transmitting a biologic signal to lymphocytes.[3-5] A 75-kda chain (p75) is associated with the 55-kda chain, and together they constitute the high-affinity receptor. IL-2R have been demonstrated on activated T cells, B cells, monocytes or macrophages, and various types of lymphoma cells.[1,2,6-16] Expression of Tac is most abundant in patients with adult T-cell leukemia/lymphoma (ATL), anaplastic large cell lymphoma, hairy cell leukemia, and Hodgkin's disease (HD).[13,14]

In normal lymphocytes, the production of IL-2, as well as the expression of IL-2R, are regulated so that an optimal immune response can be achieved. In lymphoma cells, however, the expression of IL-2R is deregulated and the constant presence of large numbers of IL-2R in lymphoma cells and/or an aberration of these receptors may play a role in the pathogenesis of uncontrolled growth of these tumor cells.[1,2] In studying the significance of Tac

expression in Tac-positive lymphomas, we are particularly interested in HD, because this lymphoma is characterized by an abundant infiltrate of activated T lymphocytes, which are capable of secreting IL-2. It is conceivable that, if the IL-2R expressed by Hodgkin's neoplastic (Reed–Sternberg [H-RS]) cells is similar to the IL-2R expressed by activated T or B lymphocytes, IL-2 can add to the proliferative activity of H-RS cells. Therefore we wanted to test whether exogenous IL-2 can promote the proliferation of the cultured H-RS cells HDLM-1, HDLM-1d, and KM-H2, which are known to express IL-2R (Tac).

## Materials and Methods

### Culture of H-RS Cell and T cells

The HDLM-1, HDLM-1d, and KM-H2 cell lines were the sources of H-RS cells.[17-19] The HDLM cells were established from the pleural effusion of a 74-year-old man with the nodular sclerosing subtype of HD. The KM-H2 cells were established from the pleural effusion of a 32-year-old man with the mixed cellular subtype of HD. The phenotypes and properties of these cells have been described in detail previously.[17-19] For comparison of the effects of IL-2 on T cells and on H-RS cells, we also included in this study the human T-lymphoma/leukemia cell lines H9, HuT78, HuT102, MOLT-4, and MT-2; a rabbit T-cell line, RT; and a murine IL-2-dependent T-cell line, CTLL-2. Among these cells, MT-2, HuT102, and RT were positive for human T-cell lymphoma/leukemia virus (HTLV-1), as shown by the expression of the HTLV-1 antigens p19, p24, and gp46 in these cells. All cells were grown at 4 × 10^5 to 2 × 10^6 cells/ml in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (lot number 1111817, HyClone Laboratories, Logan, UT), 2 $\mu$mol/l (micromolar) glutamine, 50 $\mu$mol/l 2-mercaptoethanol, and 50 $\mu$g/ml gentamicin at 37° C in a humidified, 5% $CO_2$ atmosphere.[20] The viability of the cells was maintained at 90% to 95%. Viability was determined by the Trypan Blue dye exclusion test. All these cells were tested for and found to be free of mycoplasma infection.

### Expression of IL-2R in H-RS Cells and T Cells in Culture

Expression of IL-2R (p75 and p55) in H-RS cells and T cells was determined by staining of cells with anti-TU27 and anti-Tac (each at 2.5 $\mu$g/ml), respectively.[21-23] The anti-Tac antibody was provided by Dr. T. A. Waldmann (National Cancer Institute, Bethesda, MD), and the anti-TU27 antibody by Dr. K. Sugamura (Tohoku University School of Medicine, Sendai, Japan). The specificities of

these antibodies have been described in detail previously.[21-23] The staining intensity was determined either by the avidin-biotin-peroxidase (ABC) method or by immunofluorescence studies with flow cytometry.[24-26]

### Production of IL-2 by H-RS and T Cells in Culture

We evaluated the biologic activity of IL-2 in culture supernatant by using a CTLL-2 cell proliferation assay as previously described.[27] CTLL-2 cells were cultured for 36 hours in 96-well flat-bottom microtiter plates (Costar, Cambridge, MA) at 5 × 10^3/well in 0.2 ml RPMI 1640 medium containing 10% fetal calf serum (FCS) and serial dilutions of culture supernatant or recombinant IL-2 (Genzyme, Boston, MA) at 0.5, 1, 2.5, 5, and 10 units/ml. The cells were then pulsed with 0.1 $\mu$Ci of [^125I]-deoxyuridine (iododeoxyuridine, catalog number 68019; activity, 0.50 mCi/0.524 ml; ICN Radiochemicals, Irvine, CA) for an additional 4 hours. The cells were harvested onto glass fiber filters, and the incorporated [^125I]-deoxyuridine was assessed with a gamma counter (LKB Instruments Inc., Houston, TX). All tests were done in triplicate and were repeated three times.

Because phorbol ester (tetradecanoyl phorbol-13-acetate, TPA) has been shown to enhance the production of cytokines, including IL-2, in cultured T cells,[28-29] we wanted to determine in this study whether IL-2 secretion could be increased when H-RS cells were treated with TPA. The protocol we used for TPA treatment was the same as reported previously.[20] Briefly, TPA (dissolved in dimethylsulfoxide, 14 $\mu$g/ml; Sigma, St. Louis, MO) was added at a final concentration of 2 ng/ml to cultures of H-RS cells for 2 days. The culture supernatant was examined for the presence of IL-2 activity. No dialysis was attempted for removal of TPA from the supernatant because the TPA did not affect the accuracy of the CTLL-2 assay.

In addition, we used a specific monoclonal anti-IL-2 antibody (clone DMS-1, Genzyme, Boston, MA)[30-32] to examine the expression of IL-2 in cultured H-RS cells. The characterization and specificity of this anti-IL-2 antibody have been described in detail previously.[30] Both TPA-treated and TPA/phytohemagglutinin-treated (PHA, 1 $\mu$g/ml) H-RS cells were immunostained with anti-IL-2 (2.5 $\mu$g/ml) according the ABC method described above. The TPA/PHA treatment is a standard method used for enhancement of the production of IL-2 in T lymphocytes.

### Effects of IL-2, Anti-IL-2, and Anti-Tac on the Growth of Cultured H-RS Cells and T Cells

We added IL-2, anti-IL-2, anti-Tac, or anti-TU27 to cultures to determine the effects of these reagents on the prolifera-

tion of H-RS cells and of T cells. These cells were cultured in 96-well flat-bottom microtiter plates (Costar, Cambridge, MA) at 1 × 10⁴/well in 0.2 ml RPMI 1640 medium containing 10% FCS. Recombinant IL-2 was added at concentrations of 0.1 to 100 U/ml, and the antibodies were added at 0.5 to 10 µg/10⁶ cells. Each treatment was carried out for 6 days.

We determined the effects of IL-2, anti-IL-2, and anti-IL-2R on the proliferation of H-RS cells and T cells by measuring the capacity of these cells to take up isotope-labeled deoxyuridine and by noting the increase in the numbers of cells in the S and G$_2$/M phases of the cell cycle.[17] To measure the nucleic acid uptake, we pulsed cells with [$^{125}$I]-deoxyuridine for 4 hours. The cells were then harvested and their activity was counted as described above. For DNA cycle analysis, we examined the nuclear staining by using propidium iodide (50 µg/ml) and flow cytometry, as previously reported.[17] The numbers of cells in control and IL-2- or antibody-treated cultures were monitored with a Coulter counter (Coulter Electronics, Inc., Hialeah, FL).

### Effects of IL-2 and Anti-Tac on the Regulation of Tac Antigen in Cultured Cells

We studied whether the expression of Tac antigen on the surface of H-RS cells and MT-2 cells could be regulated by the presence of IL-2 in the culture medium. The extent of regulation at various time points was examined in an immunoperoxidase assay on cytospin smears or by an immunofluorescence assay with the Ortho Fluorocytograf 50H, as previously described.[24] To detect the regulation of Tac expression, we used a high concentration of IL-2 (50 to 200 units/ml), as previously suggested.[33] Anti-tac was used at a concentration of 5 µg/ml.

### Expression of IL-2 mRNA in Cultured H-RS Cells

A Quick-Blot Kit (Schleicher & Schull, Keene, NH) was used for the preparation of mRNAs from control and TPA-treated HDLM-1 and KM-H2 cells, as described previously.[31] For Northern blots, 20 µg of total RNA or 0.1 to 1 µg of polyA+ RNA was used per lane. The RNA was treated with glyoxal at 50° C for 30 minutes before electrophoresis on a 1.2% agarose gel with phosphate buffer, pH 6.8. The RNA was transferred to a Zeta-probe membrane (BioRad) or to nitrocellulose paper (BA 83, 0.2 µm) according to procedures already described.[34] The filters were baked, prehybridized in a solution containing 3% dry milk, 6× SSC, 5× Denhardt's solution, 0.5% SDS, 0.01 mol/l (molar) EDTA, and 100 µg/ml denatured herring sperm DNA, and then hybridized overnight in the same solution containing a $^{32}$P-labeled IL-2 probe provided by Dr. K. J. Hardy (Baylor College of Medicine, Houston, TX). pBR322 containing no cloned fragments served as the negative control. The IL-2 probe has been shown to hybridize with a 0.85-kb mRNA species from PHA/TPA-stimulated (PHA, 1 µg/ml and TPA, 10 ng/ml) HuT78 cells.[35]

### Expression of IL-2R and IL-2 in H-RS Cells in Tissues

We used the ABC technique, as above, to examine the expression of IL-2 and IL-2R in H-RS cells in frozen lymph node sections from 10 patients with HD. These included seven sections from patients with the nodular sclerosis and three from patients with the mixed cellularity form of HD. The diagnosis of HD in each of these cases was confirmed by the expression of CD30, but not of other T- or B-cell markers.[14,20] As a control for staining specificity, we replaced monoclonal antibodies (MAbs) with mouse ascites fluid or with mouse Ig of identical subclass to anti-IL-2 and IL-2R in equivalent amounts.

### Results

### Expression of IL-2R in T Cells and H-RS Cells

The two types of HTLV-1-positive human T cells (MT-2 and HuT 102) expressed p55 and p75 IL-2R that could be detected with the MAbs anti-Tac and anti-TU27. The cells of the other three human T-cell lines, HuT78, H9, and MOLT-4, which we have cultured in our laboratory, did not express Tac or TU27, or expressed them in less than 1% of the cells. All three of these Tac/p75-negative cell types were HTLV-1 antigen negative. The anti-TU27 and Tac did not cross react with the IL-2R associated with rabbit or murine T cells; therefore we could not determine the expression of IL-2R in RT and CTLL-2 cells.

HDLM-1 and -1d cells expressed Tac with a staining intensity comparable to or greater than that in MT-2 and HuT102 cells. Expression of Tac was weak in KM-H2 cells, but the staining intensity was similar to that observed in activated B and T cells or in B-lymphoma/leukemia cells. The p75 IL-2R was absent in all three lines of cultured H-RS cells.

### Production of IL-2 by H-RS and T Cells

Cultured T cells and H-RS cells did not secrete IL-2, or secreted undetectable amounts, as determined by the CTLL-2 proliferation assay. Induction with TPA did not
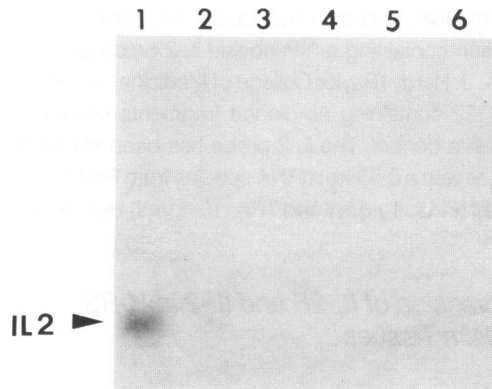
**Figure 1.** *Northern blot analysis of mRNA transcripts of PHA/ TPA-treated (lane 1) and unstimulated (lane 2) HuT78 cells, TPA-treated (lane 3) and unstimulated (lane 4) HDLM-1 cells, and TPA-treated (lane 5) and unstimulated (lane 6) KM-H2 cells with IL-2 cDNA probe. HuT78 cells, but not H-RS cells, could be induced to produce IL-2 transcript (arrow in lane 1).*

promote the production of IL-2 in any cell type. The lack of IL-2 production was also confirmed by the absence of IL-2 mRNA and IL-2 protein, as determined by Northern blot hybridization (Figure 1) and immunoperoxidase staining in control and/or TPA/PHA-treated H-RS cells. The production of IL-2 was detected in TPA/PHA-treated HuT78 cells, which we used as positive controls (Figure 1).

## Effects of IL-2, Anti-IL-2, and Anti-IL-2R on the Growth of H-RS Cells and T Cells

Recombinant IL-2, when used at concentrations in the range of 0.1 to 100 units/ml, promoted the proliferation of IL-2R (p75/p55)-positive, but not of IL-2R-negative T cells (Table 1 and Figure 2). The dose of IL-2 required for a maximal effect on IL-2R-positive T cells was approximately 10 to 20 U/ml. There was an approximately four- to sixfold increase in nucleic acid uptake in these cells (Figure 2, panel B) after IL-2 treatment. An exception was CTLL-2, which is an IL-2-dependent cell line. The increase in nucleic acid uptake in IL-2-treated CTLL-2 cells was more than 100-fold. The effect of IL-2 in all types of cells reached a plateau 2 days after treatment and was maintained for 4 to 6 days. The proliferative effect of IL-2 on T cells could be abolished when the cells were treated concomitantly with anti-IL-2.

In contrast to the Tac-positive T cells, H-RS cells did not respond to exogenous IL-2. The addition of IL-2 to cultures of H-RS cells, with or without TPA treatment, did not promote the proliferation of H-RS cells through 6 days of treatment (Figures 2 and 3).

Addition of anti-IL-2, anti-Tac, or anti-TU27 (0.1 to 5 μg/ml) to cultures did not affect the growth of T cells or

H-RS cells. The cell cycle distribution, nucleic acid uptake, and cell numbers remained the same in both control and IL-2- or antibody-treated cultures through 6 days of incubation.

## Effects of IL-2 and Anti-Tac on the Regulation of Tac Antigen in H-RS Cells

The expression of Tac was compared in H-RS cells treated with IL-2 and those treated with anti-Tac (Table 2 and Figures 4 and 5). The addition of IL-2 to cultures of H-RS cells did not affect the intensity of Tac staining on these cells (Figure 4C). It appeared that IL-2 did not regulate, or only minimally regulated, the expression of Tac. A control study of MT-2 cells, however, confirmed that treatment with IL-2 decreased the expression of Tac during the first 2 to 4 hours of incubation. The average staining intensity of IL-2-treated MT-2 cells was approximately 30% lesser than that in cells without treatment.

Anti-Tac failed to modulate the expression of Tac antigen in H-RS cells. Anti-Tac bound to the cell surface of H-RS cells for more than 2 days. When these cells were stained with additional anti-Tac, the staining intensity was the same as that of control cells (Table 2). This finding suggests that the amounts of Tac antigen remained stable despite the presence of anti-Tac; thus anti-Tac did not induce the modulation of Tac antigen. When anti-Tac–treated H-RS cells were stained with anti-mouse Ig (Figure 4D), the staining intensity of these cells was slightly lower than that observed with H-RS cells stained with both anti-Tac and anti-mouse Ig (Figure 4B). These results indicate that anti-Tac bound to the surface of H-RS cells may be slowly internalized or shed.

**Table 1.** *$^{125}I$-IUDR Uptake in Untreated and IL-2-Treated H-RS Cells and T Cells*

| Cells | $^{125}$IUDR uptake in untreated cells | $^{125}$IUDR uptake in IL-2–treated cells |
|---|---|---|
| IL-2R-positive T cells | | |
| CTLL-2 | 154 ± 16 | 8750 ± 1235 |
| MT-2 | 1408 ± 125 | 7367 ± 904 |
| RT | 3115 ± 275 | 16,184 ± 1275 |
| HuT102 | 1249 ± 136 | 4753 ± 528 |
| IL-2R-negative T cells | | |
| HuT78 | 1536 ± 167 | 1720 ± 158 |
| MOLT4 | 8820 ± 726 | 8975 ± 822 |
| H9 | 7288 ± 897 | 7480 ± 845 |
| H-RS cells (IL-2R-positive) | | |
| KM-H2 | 1045 ± 79 | 1176 ± 95 |
| HDLM-1 | 2889 ± 167 | 2905 ± 196 |
| HDLM-1d | 1653 ± 126 | 1595 ± 119 |

The dose of IL-2 used for these data was 10 units/ml; the IL-2 treatment was carried out for 2 days.
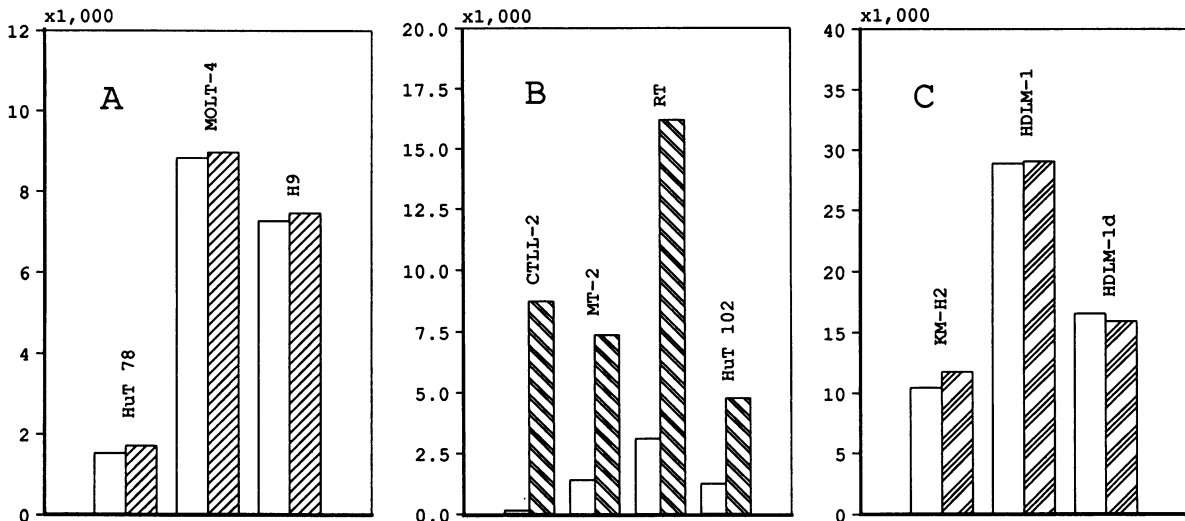
Figure 2.  Effect of IL-2 on the proliferation of IL-2R-negative T cells(A), IL-2R-positive T cells(B), and Tac-positive H-RS cells(C). There was a four- to sixfold increase in nucleic acid [($^{125}I$)-deoxyuridine] uptake by IL-2R-positive T cell 2 days after treatment with recombinant IL-2 (10 units/ml). However, despite their abundant expression of Tac, H-RS cells did not respond to exogenous IL-2. The y axis indicates the amounts of isotope taken up (cpm $\times$ $10^3$). White bars, control cultures without IL-2 treatment; shaded bars, cells treated with IL-2.

## Expression of IL-2, Tac, and p75 in H-RS Cells in Tissues

Tac antigen was detected in H-RS cells from 6 of the 10 patients with HD. In all 10 patients tested, we did not observe p75 immunoreactivity in H-RS cells. Both anti-Tac and TU27 stained approximately 5% to 20% of normal lymphoid cells in tissues. In normal or reactive lymphoid tissues (ie, tonsils), Tac and p75 were detected in 5% to 10% of lymphoid cells, as previously described.[15,22]

IL-2 was observed in H-RS cells in all of the HD patients examined. However, the number of H-RS cells positive for IL-2 varied from case to case, ranging from 30% to 75%. Furthermore, in cases positive for both IL-2 and Tac, the expression of these two proteins was not necessarily in the same population of H-RS cells. Approximately 15% to 25% of lymphoid cells, including germinal center B cells and histiocytes, were positive for IL-2. The staining in both lymphoid cells and H-RS cells had a localized granular distribution pattern (Figure 6).

Figure 3.  DNA cycle analysis of untreated H-RS cells(A, C, and E) and IL-2-treated H-RS cells (B, D, and F). The patterns remained very similar, without a significant increase in cells in $G_2/M$ and S phases (region 2), after addition of IL-2. Region 1 comprises cells in $G_0/G_1$ phase. The x axis represents the staining fluorescence intensity, and the y axis, the number of cells stained (linear scale). A, B: KM-H2 cells; C, D: HDLM-1d cells; E, F: HDLM-1 cells.
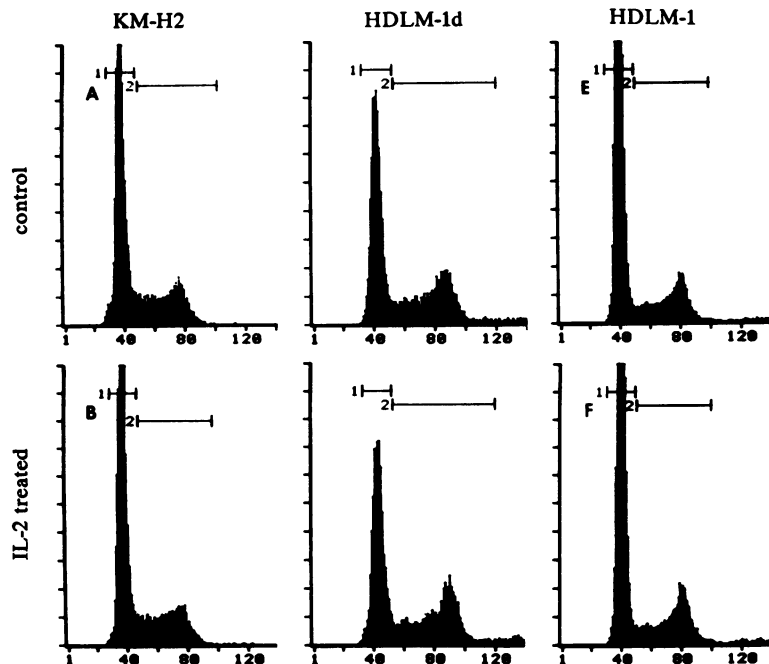
**Table 2.** *Effects of IL-2 and Anti-Tac on the Expresion of IL-2 Receptor in H-RS Cells*

|  | Stained with | Control<br>None | Control<br>p + s* | IL-2 treated<br>p + s | Anti-Tac<br>treated<br>p + s | Anti-Tac<br>treated<br>s only |
|---|---|---|---|---|---|---|
| HDLM-1 | Staining intensity† | 1.5 ± 0.2 | 528  ± 25 | 531  ± 22 | 527  ± 27 | 505  ± 32 |
|  | % positive cells | <0.1 | 98.5 ±  1.3 | 97.9 ±  1.3 | 98.2 ±  1.1 | 93.5 ±  2.7 |
| KM-H2 | Staining intensity | 1.5 ± 0.3 | 118  ± 11 | 119  ± 8 | 113  ± 8 | 77  ± 12 |
|  | % positive cells | <0.5 | 35  ± 2.5 | 35.5 ±  2.4 | 33.8 ±  1.6 | 24  ± 2.2 |

* p, primary antibody, anti-Tac; s, secondary antibody, fluorescein-conjugated rabbit anti-mouse Ig.
† The staining intensity was determined as the mean channel of fluorescence intensity of stained cells (+SD); cells positively stained were expressed as percentage + SD.

## Discussion

The cultured H-RS cells that we studied, HDLM-1, HDLM-1d, and KM-H2, expressed various amounts of p55 IL-2R (Tac). The more abundant expression was noted in HDLM-1 and HDLM-1d, whereas KM-H2 expressed lesser amounts of Tac. An additional H-RS cell line, L428, was previously reported not to express Tac, but two others (L540 and L591) were Tac positive.[9] A heterogeneous pattern of p55 IL-2R expression is also a general property of H-RS cells in tissues.[10,14] In contrast, the p75 IL-2R was uniformly absent from H-RS cells in culture and in tissues.

The absence of p75 from the surface of H-RS cells renders the cells incapable of forming a high-affinity IL-2R. This may explain the fact that the addition of IL-2 to cultures did not promote the proliferation of H-RS cells, despite the abundant expression of Tac in these cells. This also explains why IL-2 failed to down modulate the expression of p55 IL-2R in H-RS cells.

The properties of the IL-2R associated with H-RS cells are different from those identified with the cultured T cells that we tested. Many of these T-cell lines, especially those infected with HTLV-1, possess considerable numbers of p55 and p75 IL-2R.[2] Because a high-affinity IL-2R is formed in these cells, it was not an unexpected finding that these human T cells (ie, MT-2, HuT102) and the rabbit T cells (RT) could be stimulated to proliferate when exogenous IL-2 was added to the incubation medium. This was demonstrated by the fact that a proliferation index of 4.0 to 6.0 was observed for IL-2-treated MT-2, HuT102, and RT cells. Furthermore, it has been reported that binding of IL-2 to human T cells (eg, HuT102) results in cointernalization of both p55 and p75, whereas binding to p55 alone does not result in internalization of the IL-2-p55 complex.[33,36,37] This would explain why exogenous IL-2 can down modulate the expression of p55 IL-2R in MT-2 cells, as shown in this study. In contrast, the consistent expression of p55 on H-RS cells through the course of IL-2 treatment (Table 2 and Figures 4 and 5) is in accord with the absence of p75 in cultured H-RS cells.

It should be mentioned that IL-2 plays a pivotal role in B-cell proliferation.[1,2] Treatment of activated B cells, or of

Tac-positive B-leukemia/lymphoma cells in short-term culture, with IL-2 results in an active proliferative response.[38-40] The pattern of response in B leukemia/lymphoma cells[40] is very similar to that of CTLL-2, which we observed in this study. Because both T/B-lymphoma cells and H-RS cells are neoplastic, one would not expect the production or expression of IL-2 and of IL-2R to be under entirely the same regulatory mechanism found in normal T and B cells. For example, in HTLV-1–positive T-cell lines, the expression of IL-2R is deregulated, and the number of IL-2R synthesized is usually 5 to 10 times greater than that present on maximally activated normal T cells.[1,2] Furthermore, although p55 and p75 are usually coexpressed in most T- or B-lymphoma cells, p55 and p75 expression
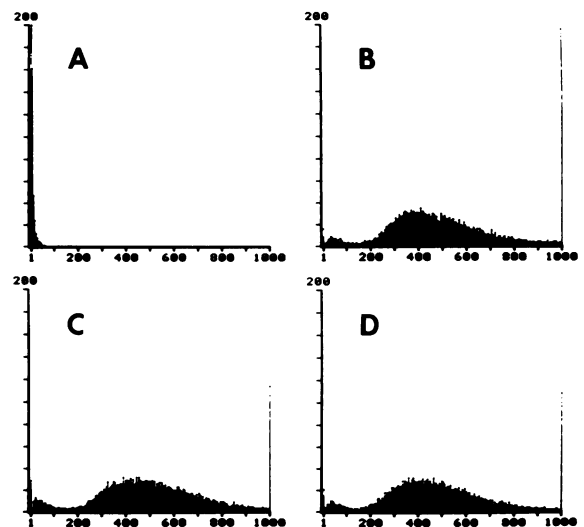


**Figure 4.** *Effects of IL-2 and anti-Tac on expression of Tac in HDLM-1 cells. Cells were pretreated with IL-2 (100 units/ml) (C) or anti-Tac (5 µg/ml) (D) for 2 days. The cells were extensively washed and stained as follows.* **A:** *Staining (negative) control; cells were treated with fluorescein-conjugated rabbit anti-mouse Ig only.* **B** *and* **C:** *Cells were stained with anti-Tac (2.5 µg/ml), followed by fluorescein-conjugated rabbit anti-mouse Ig (1:100). The staining intensity of anti-Tac in C did not differ significantly from that in B (positive control).* **D:** *Cells were stained with fluorescein-conjugated rabbit anti-mouse Ig. The staining intensity of cells in D was slightly weaker than that of cells in B. When cells in D were stained with anti-Tac; however their staining intensity was comparable to that of cells in B (not shown). The x axis represents the intensity of fluorescence, and the y axis, the number of cells stained.*
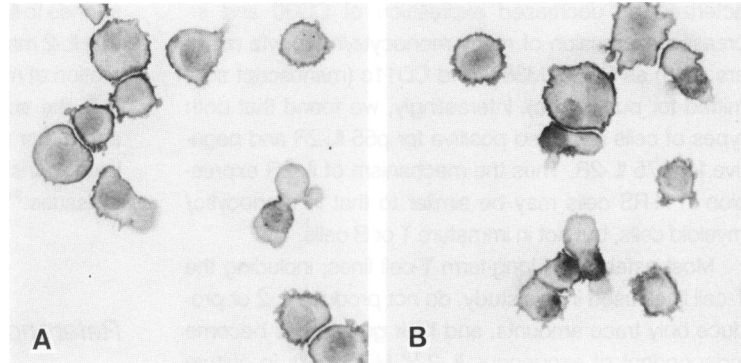
**Figure 5.** *Effect of IL-2 on expression of Tac in HDLM-1 cells.* **A:** *Control HDLM-1 cells received no IL-2 treatment.* **B:** *Cells were pretreated with IL-2 (100 units/ml) for 2 days. Both types of cells were stained with anti-Tac (2.5 μg/ml). The staining intensities in* **A** *and* **B** *were very similar.*

may be dissociated in some B-/T-lymphomas,[40,41-45] because the regulation of the genes for p55 and p75 is under separate control.[3-5] Nevertheless, the uniform absence of p75 IL-2R in H-RS cells in tissues points to an interesting property of H-RS cells.

A nonfunctional IL-2R (p55 without p75) can usually be observed in immature pro-B and pro-T cells in bone marrow, in immature thymocytes ($CD4^-/CD8^-$), as well as in monocytic/myeloid cell lines.[6-8,46-50] H-RS cells differ

from immature B or T cells by the absence of pre-B- or pre-T-cell markers. Also, H-RS cells do not have a lymphoblastoid (immature lymphocyte) appearance.[17,20] On the other hand, H-RS cells have functional properties similar to those of histiocytes and interdigitating reticulum cells.[14,17,19,34,51-54] The cultured H-RS cells, HDLM-1 and KM-H2 can be induced to differentiate along the histiocytic pathway on treatment with phorbol ester, retinoic acid, and extracellular matrix. The induced cells are char-
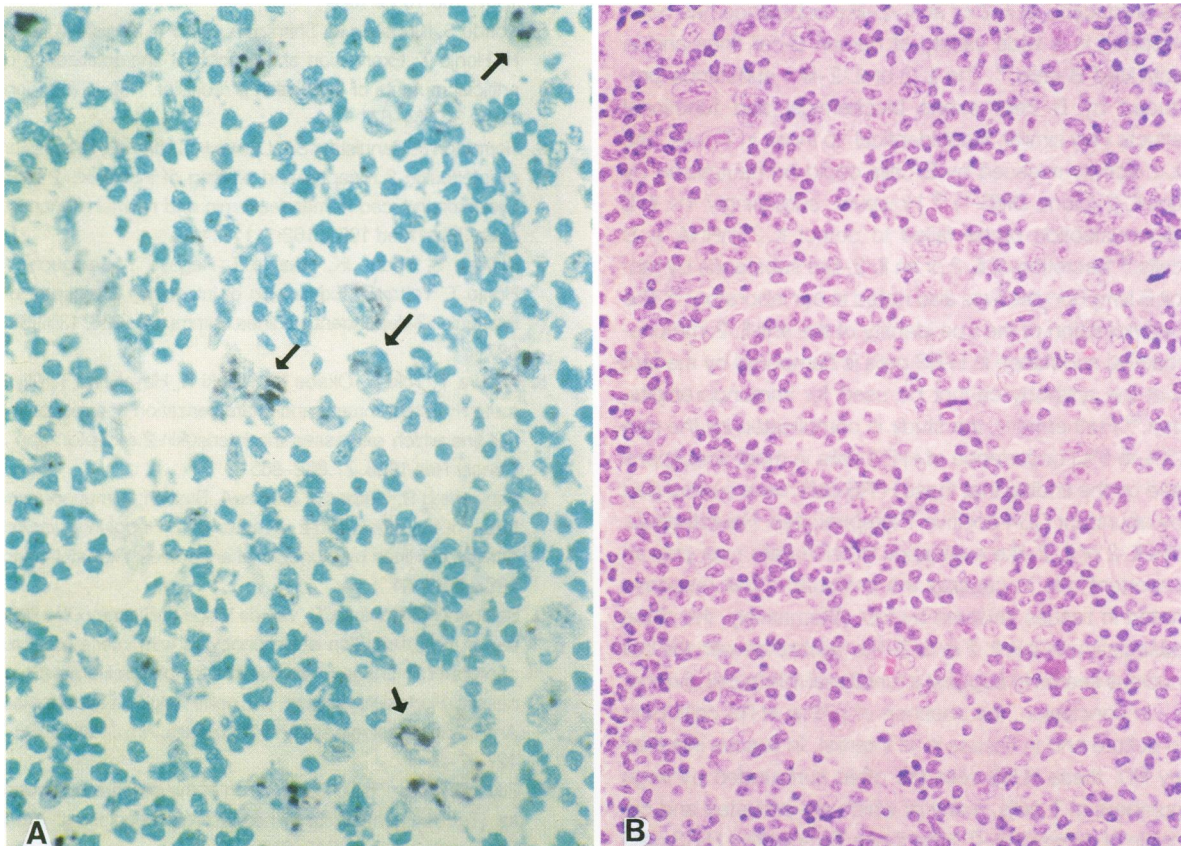


**Figure 6.** *Expression of IL-2 in H-RS cells in tissue.* **A:** *Note the granular staining in approximately 10% to 15% of T lymphocytes and in H-RS cells (arrows).* **B:** *When a paraffin section of the same tissue was stained with hematoxylin and eosin, the H-RS cells were clearly visible.* **A:** X400; **B:** X250.

acterized by decreased expression of CD30 and increased expression of many monocyte/histiocyte markers, such as CD68, M387, and CD11c (manuscript submitted for publication). Interestingly, we found that both types of cells remained positive for p55 IL-2R and negative for p75 IL-2R. Thus the mechanism of IL-2R expression in H-RS cells may be similar to that in monocytic/myeloid cells, but not in immature T or B cells.

Most established long-term T-cell lines, including the T-cell lines used in this study, do not produce IL-2 or produce only trace amounts, and their growth has become independent of exogenous IL-2.[1,2] H-RS cells in culture also did not produce IL-2, and the addition of neutralizing anti-IL-2 antibody to cultures did not affect the growth of H-RS cells. It thus appears that cultured H-RS cells can grow independently of IL-2. Because a functional assay on H-RS cells isolated from tissues is complicated by the presence of contaminating T lymphocytes, we used a monoclonal anti-IL-2 antibody and immunoperoxidase staining in this study to detect the expression of IL-2 in frozen sections. Staining was noted in 15% of T lymphocytes, and also in H-RS cells, some histiocytes, and B cells, especially those in germinal centers.

The staining of B cells by anti-IL-2 is not surprising, as production of IL-2 or an IL-2–like substance has been reported in a wide variety of human B-cell lines and in activated B cells.[32] The IL-2 or IL-2–like substance produced by B cells exerts biochemical and functional effects very similar, if not identical, to those in T cells. The IL-2 activity released by B cells could be neutralized by anti-IL-2 (clone DMS-1).[32] However, surprisingly the IL-2 oligonucleotide probe failed to detect IL-2 mRNA in B cells.[32] Secretion of IL-2 or an IL-2–like substance by rabbit B lymphocytes was also noted in our laboratory.[55] The determination of the true identity of the anti-IL-2 immunoreactive substance in H-RS cells is hampered by the fact that the antibody cannot be used for immunoprecipitation.[30] Furthermore, the study is complicated by the rare (2% to 5%) occurrence of H-RS cells in most specimens and by the finding that these H-RS cells were surrounded by abundant CD4-positive T lymphocytes. Nevertheless, this IL-2 or IL-2–like substance in H-RS cells may play an important role in causing the profound T-cell response noted in tissues involved by HD. Alternatively, the close association of this IL-2 or IL-2–like substance with the cell nucleus seems to suggest a functional role in cell division.

In conclusion, the absence of p75 in H-RS cells both in culture and in tissues is of interest. In tissues involved by HD, these H-RS cells are often surrounded by large numbers of T cells, many of which are activated and capable of releasing IL-2. How these Tac-positive H-RS cells respond to IL-2 is important for the progression of the disease and for the prognosis of the patients. The absence of p75 in most H-RS cells in tissues and the lack of response to IL-2 by Tac-positive cultured H-RS cells indicate that IL-2 may not have any significant impact on the proliferation of most H-RS cells in tissues. However, the IL-2 or IL-2–like substance, as well as other cytokines (ie, IL-1 and tumor necrosis factor) secreted by H-RS cells could be responsible for the profound T-cell reaction that occurs in tissues.[34,54,56]

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