Brief Definitive Report

STABLE EXPRESSION OF cDNA ENCODING THE HUMAN INTERLEUKIN 2 RECEPTOR IN EUKARYOTIC CELLS

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Interleukin 2 (IL-2 or T cell growth factor) is a 15,500 M_r glycoprotein critically involved in the development of the normal immune response (1, 2). IL-2 acts through specific interactions with membrane receptors expressed on the surface of activated but not resting T cells (3, 4). Using the anti-Tac monoclonal antibody (5–7), we purified human IL-2 receptor from HTLV-I (human T lymphotrophic virus I)-infected HUT 102B2 leukemic T cells, determined its NH_2-terminal sequence, and isolated cDNAs encoding the protein (8). Other investigators, using a different HTLV-I-infected cell line (9) and a different anti-IL-2 receptor antibody (10), have reported similar results. Previously (11–14), it was demonstrated that HUT 102B2 IL-2 receptors were ~5,000 daltons smaller than the receptors on normal activated T cells, at least in part reflecting differences in posttranslational processing (12).

Recently (15), radiolabeled IL-2-binding assays with activated T cells have demonstrated the presence of both high and low affinity IL-2 receptors that are indistinguishable in radiolabeled anti-Tac–binding assays. While the molecular basis for these affinity differences remains unresolved, the growth-promoting effects of IL-2 appear to be mediated by interaction of IL-2 with the high affinity receptor (3, 15).

Using a cotransfection technique and an SV40 expression vector, we now report stable expression in mouse L cells of an HUT 102B2–derived IL-2 receptor cDNA. Our findings indicate that: (*a*) the aberrant size of the HUT 102B2 receptor is recapitulated in these transfected L cells, (*b*) exogenous IL-2 does not augment the proliferation of transfected L cells, and (*c*) the expressed receptors exclusively exhibit a low apparent binding affinity for human IL-2.

Materials and Methods

Radiolabeled Probes. Purified monoclonal anti-Tac (anti–human IL-2 receptor antibody) was radiolabeled with tritium as previously described (16). Jurkat IL-2 was biosynthetically labeled with [^{3}H]leucine and [^{3}H]lysine and purified (3, 17). Two independent preparations of [^{3}H]IL-2 with specific radioactivities of 4.49×10^5 dpm/pmol and 1.52×10^4 dpm/pmol were used to measure, respectively, high and low affinity receptors (15).

Construction of pcEXV-1-IL-2R-3. The expression vector pcEXV-1, which contains the SV40 early promoter and enhancer sequences, was the gift of Drs. Ron Germain and Jim Miller, National Institutes of Health. The 2,335 base pair (bp) cDNA insert of pIL-2R-3 (8) was ligated into the EcoRI site of pcEXV-1, and plasmids with correctly oriented inserts were isolated.

Transfection of Tk⁻ L Cells. Using calcium phosphate precipitation, thymidine kinase–deficient (Tk^-) murine L cells were cotransfected with pcEXV-1-IL-2R3 DNA (5 μg/plate), pUC8-Tk plasmid DNA (60 ng/plate), and high molecular weight carrier DNA (15 μg/plate) (18). On day 2 of culture, medium containing hypoxanthine (10^{-4} M), aminopterin (4×10^{-7} M), and thymidine (1.6×10^{-5} M) (HAT) was added. After 10–14 d of culture, HAT-resistant colonies were isolated and expanded.

Analysis of Receptor Structure and Function. L cells were removed from tissue culture flasks by incubation in phosphate-buffered saline containing 0.5 mM EDTA, for 15 min at 37°C. Surface iodination and anti-Tac immunoprecipitation were performed as previously described (6). Potential IL-2-induced proliferation of L cell transfectants was evaluated by measuring [^{3}H]thymidine incorporation 24 and 48 h after addition of purified Jurkat IL-2 (6.5 pM, 6.5 nM, and 200 nM final concentrations). Radioreceptor binding assays using [^{3}H]anti-Tac and [^{3}H]IL-2 were performed as previously described (3, 15, 16).

Results

The expression vector pcEXV-1-IL-2R3 is depicted in Fig. 1, *left*. After chromosomal integration, this vector permits stable expression of cDNA under the control of the SV40 early promoter and enhancer sequences. After transfection, seven different HAT-resistant L cell transfectants, designated L-TRANS 1–7, were chosen for detailed analysis. Each of these cell populations, but not nontransfected L cells, expressed human IL-2 receptors as measured both by the binding of [^{3}H]anti-Tac and indirect immunofluorescence with anti-Tac (80–90% of cells were positive). The level of IL-2 receptor expression, however, varied significantly among the L cell colonies (1,500–18,000 receptors per cell), with L-TRANS 3 consistently displaying the greatest number of receptors.

To characterize the IL-2 receptors expressed, we radiolabeled proteins on the surface of L-TRANS 3 and HUT 102B2 cells with Na[^{125}I], and immunoprecipitated with anti-Tac or UPC-10 (a control murine IgG2a$_\kappa$ monoclonal antibody)

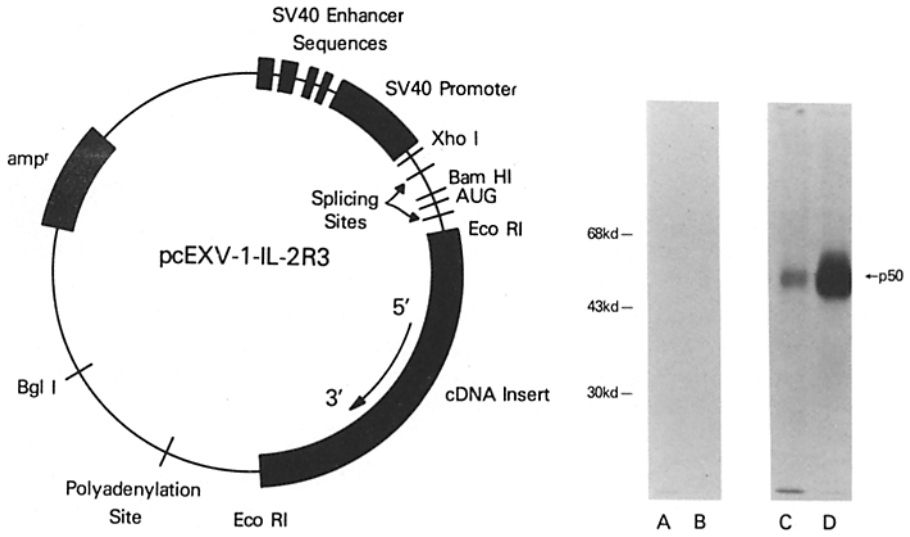

FIGURE 1. (*Left*) Schematic of the 3.0 kb pcEXV-1-IL-2R3 expression vector used for cotransfection. (*Right*) Surface iodination and immunoprecipitation of L-TRANS 3 and HUT 102B2 cells. (*A*) L-TRANS 3–UPC-10; (*B*) HUT 102B2–UPC-10; (*C*) L-TRANS 3–anti-Tac; (*D*) HUT 102B2–anti-Tac.

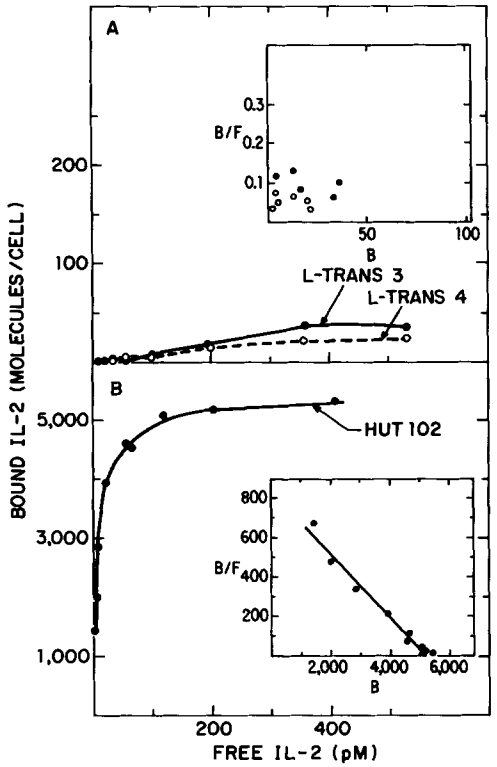

FIGURE 2. Measurement of high affinity IL-2 receptor expression in L-TRANS 3 and L-TRANS 4 cells (*A*) and HUT 102B2 cells (*B*). Scatchard conversion of the binding data is shown as an inset in each panel.

(Fig. 1, *right*). Anti-Tac, but not UPC-10, identified IL-2 receptors on L-TRANS 3 cells that were essentially identical in size (M_r 50,000) to the receptors present on HUT 102B2 cells, but ~5,000 daltons smaller than the receptors isolated from lectin-activated, normal peripheral blood lymphocytes (11–14). Despite the presence of IL-2 receptors, purified IL-2 (final concentrations, 6.5 pM, 6.5 nM, or 200 nM) did not augment [^{3}H]thymidine incorporation at 24 or 48 h in any of the L cell transfectants. These preparations of IL-2, however, did promote maximal proliferation of a cloned, IL-2-dependent murine, cytotoxic T cell line (CTLL-2), similarly exposed to EDTA.

Using purified [^{3}H]IL-2, Robb, Greene, and Rusk (15) have recently identified at least two affinity classes of IL-2 receptors on lectin-activated T cells and HTLV-infected leukemic T cell lines. In contrast to IL-2, the anti-Tac antibody bound equivalently to both affinity classes of receptors and thus could not be used to distinguish these binding site populations. The less numerous high affinity receptors (K_d [dissociation constant] 10^{-11} M) appear to mediate the growth-promoting response to IL-2 (3, 15), while the more numerous low affinity receptors (K_d 10^{-8} M) have, as yet, no defined biological function. In the presence of 100 pM free IL-2, neither L-TRANS 3 nor L-TRANS 4 cells displayed significant numbers of high affinity IL-2-binding sites (≤10 receptors per cell) (Fig. 2*A*). In contrast, at the same IL-2 concentrations, HUT 102B2 cells

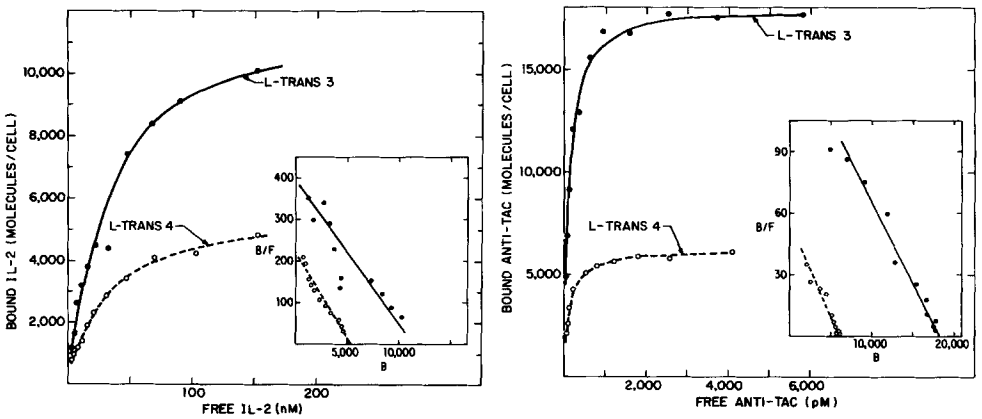

FIGURE 3. (*Left*) Measurement of low affinity IL-2 receptor expression in L-TRANS 3 and L-TRANS 4 cells. Simultaneous studies with HUT 102B2 cells demonstrated 82,000 low affinity receptors with a K_d of 2.88×10^{-8} M. Scatchard conversion of the binding data is shown in the inset in both panels. (*Right*) Measurement of anti-Tac binding sites on L-TRANS 3 and L-TRANS 4 cells. Simultaneous binding studies with HUT 102B2 cells demonstrated 185,000 receptors per cell with a K_d of 1.5×10^{-10}.

TABLE I

IL-2 Receptor Number and Affinity

Cell type	IL-2 Binding*				Anti-Tac binding‡		High affinity IL-2-binding sites as a percentage of anti-Tac sites
	High affinity		Low affinity		Sites per cell	K_d	
	Sites per cell	K_d	Sites per cell	K_d			
		pM		*pM*		*pM*	%
HUT 102B2	5,150	6.1	82,000	28,000	185,000	150	2.8
L-TRANS 3	≤10	—	11,000	28,800	18,000	130	≤0.06
L-TRANS 4	≤5	—	5,000	24,200	6,000	110	≤0.08

* Two independent preparations of [^{3}H]leu,lys IL-2 were used to measure binding, one with a specific radioactivity of 4.49×10^5 dpm/pmol (high affinity binding), and the other, 1.52×10^4 dpm/pmol (low affinity binding). The results were first corrected for nonspecific binding.

‡ Anti-Tac antibody was radiolabeled by reductive methylation using [^{3}H]$NaBH_4$ to a specific radioactivity of 3.59×10^5 dpm/pmol.

similarly exposed to EDTA bound (K_d 6.1×10^{-12} M) ~5,100 molecules of [^{3}H] IL-2 per cell (Fig. 2*B*). With regard to low affinity IL-2 receptors, L-TRANS 3 and L-TRANS 4 cells expressed 11,000 and 5,000 sites per cell, respectively, (K_d 2.88×10^{-8} M and 2.42×10^{-8} M), compared with 82,000 sites per cell for the HUT 102B2 cell line (K_d 2.88×10^{-8} M) (Fig. 3, *left* and Table I). The small amount of specific [^{3}H]IL-2 binding that occurred on L-TRANS 3 and 4 cells at 200–400 pM free IL-2 (Fig. 2*A*) may represent a portion of the binding to these low affinity receptors. [^{3}H]Anti-Tac binding to L-TRANS 3 and L-TRANS 4 cells (Fig. 3, *right*) was 18,000 and 6,000 sites per cell, respectively, which is in general agreement with the [^{3}H]IL-2-binding data. Table I presents aggregate results from these binding studies and indicates that the L cell transfectants express a typical low affinity but essentially no detectable high affinity IL-2 receptors.

Discussion

Using an SV40 expression vector and a cDNA encoding the human IL-2 receptor, we produced several L cell transfectants that have expressed surface

IL-2 receptors for >4 mo in culture. These L cell transfectants (*a*) bound [^{3}H]-anti-Tac and displayed receptors identical in size to those on HUT 102B2 cells from which the cDNA was isolated, (*b*) failed to respond to IL-2 with increased proliferation, and (*c*) expressed low affinity but not high affinity forms of the IL-2 receptor.

These findings underscore two major points. First, transfection of the HUT 102B2–derived IL-2 receptor cDNA resulted in a display of surface receptors having the aberrant size of the IL-2 receptors present on HUT 102B2 cells (M_r 50,000 rather than 55,000). Similar results have been obtained with COS-1 monkey kidney cells in transient expression studies. While the molecular basis for the difference in HUT 102B2 receptor size appears to reside in altered posttranslational processing (12), these findings raise the possibility that a difference in the primary DNA sequence may produce this difference in processing. Alternatively, L cells and COS-1 cells may share with HUT 102B2 cells similar abnormalities of posttranslational processing of the human IL-2 receptor. Final resolution of a potential difference in primary structure of the normal and HUT 102B2 IL-2 receptor awaits complete determination of the normal IL-2 receptor gene or cDNA sequence.

Second, although expressing the Tac antigen, the transfected L cells did not respond to purified IL-2 with augmented proliferation. This lack of responsiveness could reflect the malignant nature of these cells or tissue-specific restrictions of IL-2 responsiveness perhaps due to the absence of an appropriate apparatus to transmit intracellular signals. However, the finding that only low affinity IL-2 receptors are expressed in these transfected cells provides an alternative explanation for the lack of augmented proliferation, since high but not low affinity, IL-2 receptors appear to mediate IL-2-induced growth. At present, the molecular basis for differences in IL-2 receptor affinity remains undefined. Possible mechanisms that might produce receptors with high affinity for IL-2 include (*a*) formation of a receptor complex and (*b*) posttranslational modification of the receptor protein. Alternatively, it is possible that the cDNA isolated from the HUT 102B2 cells encodes only the low affinity IL-2 receptor.

Additional study of the structural and functional differences in the high and low affinity forms of the IL-2 receptor is required. The availability of a cell population that expresses only low affinity receptors, however, should help elucidate these differences and provide possible insights into the mechanism(s) of signal transmission used by the IL-2 receptor.

Summary

Human interleukin 2 (IL-2) receptor cDNA derived from HUT 102B2 cells was stably expressed in murine L cells. These L cell transfectants (*a*) displayed surface receptors of the aberrant size of the IL-2 receptors on HUT 102B2 cells, (*b*) did not respond to exogenous IL-2 with augmented proliferation, and (*c*) expressed low affinity but not high affinity receptors for IL-2.

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