The Human Receptor for T-cell Growth Factor

EVIDENCE FOR VARIABLE POST-TRANSLATIONAL PROCESSING, PHOSPHORYLATION, SULFATION, AND THE ABILITY OF PRECURSOR FORMS OF THE RECEPTOR TO BIND T-CELL GROWTH FACTOR*

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The T-cell growth factor (TCGF) receptor on phytohemagglutinin-activated normal peripheral blood T-cells is characterized as a glycoprotein with an apparent M, of 55,000 that contains N-linked and O-linked carbohydrate with only approximately 33,000 daltons of peptide structure (p33) as evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. There are two N-linked glycosylated intermediate precursor forms (apparent M, = 35,000 (p35) and 37,000 (p37)). This receptor differs from the TCGF receptor on HUT-102B2 cells (apparent M, = 50,000) because of differences in post-translational processing. Experiments with the carboxylionophore monensin demonstrate blockade of the transition of the p35 and p37 receptor precursor forms to the mature receptor, presumably secondary to inhibition of Golgi-associated receptor processing. We identify the primary translation product of TCGF receptor mRNA as intermediate in size between the p33 and the p35/p37 forms. We further demonstrate that the p33, p35, and p37 precursor forms, but not the primary translation product, are all capable of binding TCGF. Thus, the removal of the signal peptide and/or conformational changes of the primary translation product are necessary for ligand binding; however, the extensive post-translational modifications are not. Lastly, we demonstrate that at least some TCGF receptors are phosphorylated and sulfated, and that TCGF receptors on phytohemagglutinin-activated normal T-cells are more heavily sulfated than those on HUT-102B2 cells.

T-cell growth factor (also known as interleukin-2) is a 14,800-Da glycoprotein required for T lymphocyte proliferation following exposure to antigen or mitogen (1–3). TCGF has permitted the cloning and prolonged growth of human T-cells in vitro (4, 5). High affinity specific membrane receptors for TCGF have been demonstrated on activated T-cells (6). Monoclonal anti-Tac antibody, prepared by Uchiyama and co-workers (7, 8), has been shown to recognize the human TCGF receptor (9–11) and is capable of producing a functional blockade of this receptor in vitro (12). As evaluated by reactivity with anti-Tac, all HTLV-infected T-cell lines we have studied express large numbers of TCGF receptors. HTLV is a type C retrovirus etiologically associated with a cutaneous T-cell lymphoma/T-cell leukemia known as Adult T-cell Leukemia (13, 14). The TCGF receptor on HTLV-infected Hut-102B2 cells has been identified as a glycoprotein with apparent M, of 50,000 that contains intrachain disulfide bonds and has a PI of 5.5–6.0. The receptor consists of approximately 33,000 Da of peptide backbone that is sequentially processed through at least two intermediate forms to a mature form containing both N- and O-linked carbohydrate and sialic acid (10).

We now report that some HTLV-infected cells display TCGF receptors that differ slightly in their migration on SDS gels from the normal receptors on PHA-activated peripheral blood T-cells obtained from normal donors. Further, we investigate the biochemical basis for the difference between the receptors on HUT-102B2 cells and PHA-activated T-cells, identify the primary translation product for Hut-102B2 mRNA, provide evidence that both receptor forms are phosphorylated and sulfated, and demonstrate that precursor forms of the receptor can bind TCGF.

EXPERIMENTAL PROCEDURES

HTLV-infected cells used in these studies included cell lines recently established from the peripheral blood of 3 ATL patients evaluated at the National Institutes of Health (PL/P6, OB/P1, and CF/P1), cells from long standing cloned lines from the blood and lymph node from another patient with ATL (CTC-2 and Hut-102B2), and cells from an HTLV-transformed cord blood lymphocyte line (C6/MJ). Normal activated peripheral blood T-cells were prepared by Ficoll/Hyphae centrifugation, stimulation for 72 h with 0.5 μg/ml E-PHA (Burroughs Wellcome), and then maintained in long term culture in RPMI 1640 medium containing 10% fetal bovine serum and 10% TCGF (Cellular Products). Biosynthetic labeling of cellular proteins with [35S]methionine (1000 Ci/mmol) and d-[3H]glucosamine (32.5 Ci/mmol) from New England Nuclear, extraction of cellular proteins with Triton X-100-containing buffer, and immunoprecipitation of the cellular extracts with anti-Tac, OKT4, or OKT3, and analysis on SDS-polyacrylamide gels were performed as previously described (9).

Two-dimensional gels were performed essentially as described by O’Farrell (15). The first dimension isoelectric focusing pH gradient was established with Pharmalyte 3–10 Amphiolines (Pharmacia); the second dimension was a 7.5% SDS-PAGE electrophoresed under reducing conditions.

For pulse-chase studies, cells were washed in balanced salt solution and resuspended in methionine-free media to which 0.4 mCi of [35S]methionine was added.
methionine was added for 15 min. A large excess of unlabeled methionine was then added and the chase continued for 0, 15, 30, 60, 120, or 240 min followed by membrane solubilization with nonionic detergent, immunoprecipitation with anti-Tac, and analysis by SDS-PAGE (10). In some experiments, 2 µg/ml tunicamycin was added to the media to prevent N-linked glycosylation.

The primary translation product for TCGF receptor mRNA was identified as follows: mRNA from HUT-102B2 cells or PHA-activated lymphoblasts was isolated by the method of Chirgwin et al. (17) and translated in a wheat germ lysate cell-free translation system as previously described (18); the translations were boiled in 2% SDS (19) and then immunoprecipitated with a heteroantibody to the TCGF receptor.

RESULTS

PHA-activated T-cells and cells from a variety of HTLV-positive lines were biosynthetically labeled with [3H]glucosamine and extracted, and cellular extracts were immunoprecipitated with anti-Tac and analyzed in the presence or absence of dithiothreitol on 8.75% SDS-polyacrylamide gels (Fig. 1). Both panels demonstrate a mild heterogeneity in migration of receptors on SDS gels in some HTLV-infected cells as compared to normal PHA-activated lymphoblasts.

Since [3H]glucosamine was the isotope used, these data confirm that the receptors on all of these cells are glycoproteins, as previously shown for HUT-102B2 cells (9, 10). Each receptor has an apparent M, approximately 5000 greater when electrophoresed under reducing conditions (panel a) as compared to nonreducing conditions (panel b). Thus, each of these TCGF receptors appears to contain intrachain disulfide bonds, as recently reported for HUT-102B2 cells (10).

Following lactoperoxidase-mediated cell surface iodination, the receptor size on three other HTLV-infected cell lines and on PHA blasts, HUT-102B2, CF/P1, and PL/P6 has been studied, and similar size heterogeneities have been found (20).

Since the migration on SDS-PAGE of the TCGF receptors on HUT-102B2 cells differed the most from those on PHA lymphoblasts, we compared the receptors on these two cell types in order to determine the molecular basis for the aberrancy in receptor size. As shown in Fig. 2, when anti-Tac immunoprecipitations of cellular extracts from [35S]methionine-labeled cells were analyzed on a two-dimensional gel, the HUT-102B2 receptor migrated with a slightly broader and more basic isoelectric point range (5.5–6.0) than that of the normal sized PHA lymphoblast receptor (5.4–5.7). Also present is p113, a nonglycosylated protein present in anti-Tac immunoprecipitations from both HUT-102B2 cells and PHA-activated lymphoblasts. We have previously hypothesized that this protein may be part of a receptor complex if one exists; alternatively, it may be fortuitously co-immunoprecipitated with the TCGF receptor, but not related to its structure or function.

It was possible that the difference in TCGF receptor size might be a manifestation of a general glycosylating or membrane protein processing defect in HUT-102B2 cells. We therefore
performed control immunoprecipitations using monoclonal antibodies OKT4 and OKT9 with \[^{[3]}\text{H}]\text{glucosamine-labeled}\) PHA lymphoblasts and HUT-102B2 cells. OKT4 recognizes a membrane protein primarily on helper T-cells; OKT9 is an anti-transferrin receptor antibody. As seen in Fig. 3, in contrast to the difference in apparent receptor size, which we hypothesize is mainly due to addition of O-linked carbohydrate, occurs later during the chase, consistent with a requirement for transport through the Golgi apparatus. A very similar time course of post-translational processing has been demonstrated for the low density lipoprotein receptor which also contains large amounts of O-linked carbohydrate (23).

Precursor and mature forms of PHA lymphoblast and HUT-102B2 TCGF receptors are directly compared in Fig. 5. In the left panel, cells were labeled with \[^{[3]}\text{S}\]methionine for \(30\) min in the presence of tunicamycin, extracted, and immunoprecipitated with anti-Tac, and the \(p35/p37\) precursor forms from PHA lymphoblasts (lane \(A\)) and HUT-102B2 cells (lane \(B\)) were shown to be identical in size. In the right panel, cells were labeled in the absence of tunicamycin and chased with unlabeled methionine for \(30\) min (lanes \(A\) and \(B\)) or \(4\) h (lanes \(C\) and \(D\)). The \(p35/p37\) precursor forms of the TCGF receptor precipitated by anti-Tac from PHA lymphoblasts (lane \(A\)) and HUT-102B2 cells (lane \(B\)) are identical in size. In contrast, as also shown in Figs. 1 and 2, the mature receptor forms on PHA lymphoblasts (lane \(B\)) and HUT-102B2 cells (lane \(D\)) differ in size. This figure demonstrates that TCGF receptors on HUT-102B2 cells and PHA-activated lymphoblasts differ in apparent receptor size, which we hypothesize is mainly due to addition of O-linked carbohydrate (23).

The HUT-102B2 receptor has been shown to contain approximately \(33,000\) Da of peptide that is sequentially N-linked and O-linked glycosylated with the subsequent addition of sialic acid (10). By pulse-chase analysis, tunicamycin, endoglycosidase F, and neuraminidase studies, we now show that the mature receptor size, which we hypothesize is mainly due to addition of O-linked carbohydrate, occurs later during the chase, consistent with a requirement for transport through the Golgi apparatus. A very similar time course of post-translational processing has been demonstrated for the low density lipoprotein receptor which also contains large amounts of O-linked carbohydrate (23).
**Fig. 4.** Pulse-chase studies of PHA lymphoblasts in the absence (*left panel*) or presence (*right panel*) of 2 μg/ml tunicamycin. Precipitations are done with either anti-Tac or control UPC10 monoclonal antibodies. Time of chase is on the abscissa and migration of molecular weight markers on the ordinate. Location of p33, p35, p37, and p113 is indicated. BPB, bromphenol blue.

**Fig. 5.** Precursor forms of the TCGF receptor present on PHA lymphoblasts (*left, lane A*, tunicamycin; *right, lane A*, no tunicamycin) and HUT-102B2 cells (*left, lane B*, tunicamycin; *right, lane B*, no tunicamycin), and mature receptor forms on PHA lymphoblasts (*right, lane C*) and HUT-102B2 cells (*right, lane D*).

blasts have identically sized precursor forms, but that the mature receptors differ by approximately 5,000 in apparent $M_r$ values. Also seen in this figure are bands representing actin ($M_r = 43,000$) and myosin ($M_r = 200,000$), both of which routinely are present in immunoprecipitations performed with anti-Tac.

Fig. 6 demonstrates the effects of neuraminidase and endoglycosidase F digestion of anti-Tac immunoprecipitations of cellular extracts from $[^{35}S]$methionine-labeled HUT-102B2 cells (*lanes B and C*) and PHA lymphoblasts (*lanes E and F*) compared to uncleaved receptors from HUT-102B2 cells (*lane A*) and PHA lymphoblasts (*lane D*). The immature and mature receptors on both cells are cleaved with endoglycosidase F, confirming the presence of N-linked sugar suggested by the tunicamycin studies (Figs. 4 and 5). Neuraminidase cleavage appears to result in a slightly greater change in $M_r$ in the
PHAs-activated lymphoblast mature receptor than in the
HUT-102B2 cell receptor (change in apparent M, of approxi-
mately 7,000 for PHA-activated T-cells and 5,000 for HUT-
102B2 cells). Nevertheless, the neuraminidase-cleaved PHA
lymphoblast receptor is larger than the cleaved HUT-102B2
receptor; thus, the differences in receptor size are not ex-
plained solely on the basis of differences in the degree of sialic
acid addition.

We next performed experiments designed to better char-
acterize the precursor forms of the TCGF receptor and the
role of the Golgi apparatus in their processing to the mature
form. First, we studied the effects of monensin, a carboxylic
ionophore that interferes with post-translational processing
and intracellular transport of membrane and secretory pro-
teins by interfering with terminal glycosylation and transport
of proteins through the Golgi complex (24-27). As shown in
Fig. 7, immunoprecipitations on extracts of cells cultured in
the absence of monensin demonstrate that the p35/p37 pre-
cursors seen after 0.5 h of labeling with [35S]methionine (first
lane) are processed to the mature receptor form after chasing
with unlabeled methionine for 4 h (third lane). In contrast,
in the presence of monensin (10^{-6} m), the p35/p37 precursors
seen after short term labeling (second lane) appear to be
processed to a single p37 band, but not the mature receptor,
after chasing with unlabeled methionine (fourth lane). These
experiments suggest processing of the receptor in the Golgi at
some time after the initial addition of N-linked carbohydrate
and further suggest that the p35 precursor is processed
through the p37 precursor prior to appearance of the mature
receptor.

Next, we identified the primary translation product for the
TCGF receptor. HUT-102B2 mRNA was translated in a
wheat germ lysate cell-free translation system and the prod-
ucts of translation were precipitated with preimmune serum
or with a specific heteroantiserum for the TCGF receptor
(left panel, Fig. 8). In data not shown, identically sized TCGF
receptor primary translation products were identified from
translations of mRNA from either HUT-102B2 cells or from
PHA-activated T-cells. The primary translation product (ap-
parent M, = 34,000) was intermediate in size between the p33
and p35/p37 forms, suggesting that a signal peptide is present
(right panel, Fig. 8).

We next investigated the ability of the receptor precursors
identified by anti-Tac to bind TCGF (Fig. 9). Cellular extracts
from [35S]methionine pulse-labeled PHA-activated lymphob-
lasts were incubated with TCGF Affi-Gel or anti-Tac Sephar-
ose columns, the beads were washed extensively, and the
bound proteins were eluted by boiling in SDS. Panel a shows
that p35 and p37 are readily bound to both anti-Tac Sepharose
and TCGF Affi-Gel but that neither is bound to a control
immunoglobulin column. In panel b, when cells were labeled
in the presence of tunicamycin, p33 similarly is bound to anti-
Tac Sepharose and TCGF Affi-Gel, but not to control col-
umns. These results complement results from longer labeling
experiments in which both anti-Tac Sepharose and TCGF
Affi-Gel columns recognize the identical mature receptors
(11). In data not shown, the primary translation product did
Fig. 8. Identification of the primary translation product for TCGF receptor mRNA. Left panel, immunoprecipitation of primary translation product with preimmune serum or heteroantiserum. Right panel, primary translation product (lane A), p33 (lane B), and p55/p37 (lane C).

Fig. 9. PHA-activated lymphoblasts were labeled with [35S]methionine in the absence (panel a) or presence (panel b) of tunicamycin. Panel a, cells were extracted and the extracts were passed over anti-Tac Sepharose (lane A), TCGF Affi-Gel (lane B), or a control column (lane C). Panel b, cells labeled in the presence of tunicamycin were extracted and the extracts were passed over a control column (lane A), anti-Tac Sepharose (lane B), or TCGF-Affi-Gel (lane C).

Lastly, we evaluated whether the TCGF receptors are phosphorylated and sulfated. A number of other receptors, including growth factor receptors, are known to be phosphorylated (see "Discussion") and it has been hypothesized that receptor phosphorylation may be a critical step to the transduction of a growth factor's signal into a cell. As shown in Fig. 10, we provide evidence that TCGF receptors on both PHA-activated lymphoblasts (panel A) and HUT-102B2 cells (panel B) are phosphorylated. In these studies employing intact cells labeled with [32P]orthophosphoric acid, the receptors are phosphorylated in the absence of exogenous TCGF and the phosphorylation is not obviously augmented by the addition of purified TCGF; however, as noted under "Discussion," these data do not exclude a role for TCGF in receptor phosphorylation. Further, we do not yet know whether the phosphorylation occurs on serine, threonine, or tyrosine residues. In panel b, it appears that HUT-102B2 may contain a small amount of normal sized phosphorylated receptor (the less intense band above p50). Panel b contains a moderate amount of 32P in the high molecular weight range. We have seen this in some experiments with PHA lymphoblasts and assume it is nonspecific background 32P. Unexplained high molecular weight 32P label is also evident in analogous experiments involving the insulin receptor (28). Anti-Tac routinely co-immunoprecipitates actin, myosin, and two other proteins, denoted p113 and p180 (9, 10). p113 plus p180 and/or myosin appear to be phosphorylated (panel a) and their phosphorylation is not increased when exogenous TCGF is added. As expected, actin, which is always co-immunoprecipitated nonspecifically (see, for example, Figs. 2 and 4), is not phosphorylated. It is conceivable that if the receptor were a kinase, some anti-Tac heavy chain might be phosphorylated and might appear in the same general region as the receptor. However, TCGF receptors themselves must be phosphorylated since heavy chain would not differentially migrate at 55,000 Da (receptor size on PHA lymphoblasts) and 50,000 Da (receptor size on HUT-102B2 cells).

Recently, a number of proteins have been demonstrated to contain elemental sulfate (see "Discussion"). We therefore evaluated if TCGF receptors were sulfated. Shown in Fig. 11 are the results of a sulfation experiment in which equal numbers of PHA-activated lymphoblasts and HUT-102B2 cells were labeled with [35S]sulfuric acid and immunoprecipitated with anti-Tac. Because PHA-activated lymphoblasts contain 3- to 10-fold fewer receptors/cell than HUT-102B2 cells, it is clear that on average, TCGF receptors on PHA-activated lymphoblasts must be more heavily sulfated. As
**Fig. 10.** TCGF receptors are sulfated. Panel A, PHA-activated lymphoblasts were labeled with \[^{32}P\]orthophosphoric acid, incubated without (uninduced) or with TCGF for 10 min at 37 °C; extracted, and cellular extracts were immunoprecipitated with anti-Tac or UPC10. Panel B, a similar experiment with HUT-102B2 cells: cellular extracts immunoprecipitated with UPC10 or anti-Tac following an incubation in the absence (uninduced) or presence of exogenous TCGF.

**Fig. 11.** HUT-102B2 cells and PHA-activated lymphoblasts were labeled with \[^{35}S\]sulfuric acid, extracted, and the cellular extracts immunoprecipitated with anti-Tac.

determined by both densitometry of autoradiograms and measurement of receptor-associated radioactivity eluted from isolated bands on the gel, we estimate that the amount of sulfate incorporated into the PHA lymphoblast TCGF receptors is 3- to 5-fold more per cell, or an average of about 5- to 50-fold more per receptor molecule. Although eukaryotic cells cannot convert \[^{35}S\] provided as sulfuric acid into sulfides, we confirmed that the radiolabel present in the receptor was indeed \[^{35}S\]O\(_4\), by subjecting the receptor from both cell types to acid hydrolysis (24 h in 6 M HCl at 100 °C) and then finding that greater than 90% of the counts were precipitable with barium (data not shown). As in the phosphorylation experiment, HUT-102B2 cells labeled with sulfuric acid appear to have a certain amount of normally sized receptor.

**DISCUSSION**

In this paper, we present an analysis of the structure of the TCGF receptor present on PHA-activated normal T-cells and demonstrate that there is a difference in apparent size of TCGF receptors present on these cells compared to HTLV-infected HUT-102B2 cells as evaluated by migration on SDS gels. This is of interest since HUT-102 cells are one of the prototype HTLV-I-infected TCGF receptor positive cell lines and were derived from the first patient from whom HTLV was isolated (29). Some, but not all, other HTLV-infected cell lines also have TCGF receptors that differ in apparent Mr from the normal receptors on PHA-activated lymphoblasts, albeit less than those receptors on HUT-102B2 cells, and generally by less than 2000 Da. All of these receptors are glycoproteins that contain intrachain disulfide bonds. The receptors on HUT-102B2 cells as compared to those on PHA-activated lymphoblasts appear 5000 Da smaller (Mr = 50,000 versus Mr = 55,000) and have a more basic isoelectric point (pI 5.5–6.0 versus a pI of 5.4–5.7). By tunicamycin and pulse-chase experiments, we have demonstrated that both receptors have a peptide backbone with apparent Mr = 33,000 that is initially processed, presumably co-translationally, to Mr = 35,000 and Mr = 37,000 intermediate forms, and then post-
The primary translation product has an apparent M, larger than p33 and thus contains a signal peptide. The initial processing appears to be based in N-linked glycosylation, as indicated by the time course of appearance and by tunicamycin and endoglycosidase F studies. Subsequently, the transition to the mature receptor is a monensin-sensitive event that presumably involves transport through the Golgi apparatus. Mature TCGF receptors contain sialic acid; however, the difference in size of the receptors is not based solely on the extent of addition of sialic acid. No differences in the receptor peptide backbone have been detected, but amino acid or DNA sequencing will be required to determine if any differences exist. Indeed, a point mutation at a critical amino acid could result in loss of an entire carbohydrate structure and/or sulfate or phosphate group(s), which could result in a change in the M, of the mature receptor. The key biosynthetic abnormality in the HUT-102B2 receptor appears to be based in post-translational modifications and therefore differs significantly from the aberrant form of the epidermal growth factor receptor identified in A431 epidermal carcinoma cells. In these cells, the epidermal growth factor receptor exists in two forms, including one that appears to result from altered RNA splicing with the production of a truncated peptide lacking a cytoplasmic domain (30, 31).

Recently, sulfation of a number of proteins has been described, including matrix proteins such as entactin (32), an endothelial cell basal lamina-associated glycoprotein, three glycoproteins of murine zonae pellucidae (33), and glycoproteins of the corneal epithelium (34). Only one membrane receptor, the low density lipoprotein receptor, has been reported to be sulfated (23). Sulfation has been shown to occur on either N- or O-linked carbohydrate structures (34) or on tyrosine residues (35). The overall function of sulfation and significance of its location (i.e. carbohydrate versus tyrosine-associated) is unknown. We are not yet aware of the location of the sulfate group(s) on TCGF receptors. It is of interest that the PHA-activated lymphoblast TCGF receptors appear to be more heavily sulfated than those on HUT-102B2 cells, although it is unclear whether this difference represents more sulfate on every PHA blast receptor molecule or whether only a fraction of the HUT-102B2 TCGF receptors are sulfated.

In contrast to sulfation, many growth factor receptors such as epidermal growth factor (36) and insulin (28) receptors have been shown to be phosphorylated, and these and some other receptors have been shown to have tyrosine-specific kinase activity induced by the appropriate growth factor. We do not yet know the site of phosphorylation of TCGF receptors, nor do we know whether the receptor itself may contain kinase activity as has been shown for the epidermal growth factor (37) and insulin (38) receptors. Further, we have not been able to demonstrate inducibility of receptor phosphorylation by the addition of TCGF. However, HUT-102B2 cells grow independently of exogenous TCGF and thus may always be growing in an induced fashion. These cells have been shown to endogenously synthesize TCGF (39, 40), although it is not known if they utilize it for growth. PHA-activated lymphoblasts initially produce TCGF and then are supported in cell culture in the presence of exogenous TCGF. Prior to some of the phosphorylation experiments, we have "rested" these cells in order to deplete them of TCGF. Unfortunately, this has resulted in a marked decrease in 32P uptake by the cells and no receptor could be visualized, even after the 10-min incubation with TCGF. Thus, although we have not demonstrated a role of TCGF in mediating phosphorylation of TCGF receptors, we cannot yet exclude one. Chaplin et al. (41) have previously shown that a large number of proteins in lymphocytes become phosphorylated after stimulation with PHA or other mitogens and hypothesize that such phosphorylations may play an important role in the initiation of the proliferative response. Phosphorylation of TCGF receptors may be one such key event.

The HUT-102B2 cell receptor is capable of binding TCGF as shown by 1) the presence of an acid-elutable TCGF from HUT-102B2 cells (39, 40) and by 2) direct binding of JUR- KAT [3H]TCGF to these cells (6). This is not surprising in view of the data we present from PHA lymphoblasts that the p33, p35, and p37 precursors are all capable of binding TCGF. The extensive modification of the peptide backbone of the TCGF receptor by sulfation, phosphorylation, and glycosylation to an apparent M, of 55,000 is therefore not essential to ligand binding; however, it is possible that these modifications alter affinity of binding or in other ways are essential to normal receptor function. In a different type of experiment, Stevens et al. (42) have demonstrated on intact chondrosarcoma cells that insulin binding is diminished in cells grown in the presence of tunicamycin, suggesting that N-linked carbohydrate may be important to the binding site of insulin.

The way in which TCGF receptors and/or receptor size variations relate to the uncontrolled growth of HTLV-infected cells is unknown and is especially puzzling since their growth sometimes occurs in the absence of detectable TCGF or TCGF mRNA (43). Tsudo et al. (44, 45) have demonstrated that in contrast to PHA-activated lymphoblasts, HTLV-infected cells do not modulate their receptors in the presence of anti-Tac and we have recently shown that the receptor number is uniformly very large in HTLV-infected cell lines (20). Thus, at least some of the time, HTLV infection is associated with both qualitative and quantitative abnormalities of TCGF receptors. It is possible that transformation with HTLV alone provides the stimulus for the malignant growth, perhaps by activation of a not yet identified oncogene. Alternatively, it is conceivable that the receptors on these infected cells are in some fashion themselves responsible, perhaps by providing a constant stimulatory signal (i.e. they are perhaps constantly "activated" receptors). Indeed, we have demonstrated that although anti-Tac is capable of blocking the binding of TCGF and the proliferation of TCGF-dependent cell lines, it is not capable of blocking the proliferation of those HTLV-infected, TCGF receptor-positive cell lines that are TCGF-independent. In this paper, we have identified that TCGF receptors are both phosphorylated and sulfated, and that variable post-translational processing of TCGF receptors can occur. Whether these specific modifications are critical to receptor function and/or autonomy of growth of HTLV-infected cells remains an area for further research.

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Note Added in Proof.—Since the original submission of this manuscript, Wano et al. (46) have also identified differences in the migration on SDS gels of TCGF receptors precipitated from HUT-102B2 cells and PHA-activated lymphoblasts. Furthermore, cDNAs
for the TCGF receptor have been identified, sequenced, and expressed in eukaryotic cells (Leonard et al. (47), Nikaido et al. (48)). The deduced amino acid sequence contains two potential N-linked glycosylation sites, both of which are presumably used since the p35 and p37 precursor forms described in the current manuscript are distinct N-linked precursors of mature TCGF receptors. The protein has a short cytoplasmic domain containing one serine and one threonine, which may represent the potential phosphorylation sites. There is no tyrosine residue in the cytoplasmic domain.

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