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Immunology

Characterization of the human receptor for T-cell growth factor

(disuccinimidyl suberate/crosslinking/anti-Tac monoclonal antibody/post-translational processing/interleukin 2)

WARREN J. LEONARD*, JOEL M. DEPPER*, RICHARD J. ROBB†, THOMAS A. WALDMANN*, AND WARNER C. GREENE*

*Metabolism Branch, National Cancer Institute, Bethesda, MD 20205; and †Central Research Development Department, E. I. Dupont, de Nemours and Company, Glenolden, PA 19036

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ABSTRACT **Anti-Tac monoclonal antibody has been identified as a putative antibody against the receptor for T-cell growth factor (TCGF). We now show that: (*i*) TCGF blocks 85% of ^{3}H-labeled anti-Tac binding to phytohemagglutinin-activated lymphoblasts and (*ii*) both anti-Tac and anti-TCGF immunoprecipitate a protein band that appears to represent TCGF crosslinked to its receptor on HUT-102B2 cells. In HUT-102B2 cells, the TCGF receptor is a M_r 50,000 glycoprotein with internal disulfide bond(s) and a pI of 5.5–6.0, and it represents ≈0.05% of total cellular *de novo* protein synthesis. It contains a peptide of M_r 33,000 that is processed to a mature form that includes N-linked and O-linked sugars and sialic acid.**

T-cell growth factor (TCGF or interleukin 2) is a M_r 14,800 polypeptide hormone necessary for expansion of T lymphocytes critical to the evolution of a normal human immune response (1–3). TCGF has permitted the cloning and prolonged growth of human T cells *in vitro* (4, 5). Using purified radiolabeled TCGF, Robb *et al.* (6) demonstrated high-affinity specific membrane receptors for TCGF on activated T cells. Uchiyama and co-workers (7, 8) prepared a monoclonal antibody termed anti-Tac that binds to antigen- and lectin-activated peripheral blood T cells and to all TCGF-dependent continuous T-cell lines studied but not to resting T cells, B cells, macrophages, or TCGF-independent T-cell lines. This pattern of reactivity is similar to that found for TCGF (6). We have reported that anti-Tac (*i*) blocks ≈75% of TCGF-induced DNA synthesis in a TCGF-dependent CTC line and (*ii*) blocks essentially 100% of radiolabeled TCGF binding to a human T-cell line that expresses TCGF receptors (9). Moreover, we have shown that anti-Tac blocks many TCGF-dependent T cell-mediated immune reactions (10). Therefore, we suggested that anti-Tac may recognize the human receptor for TCGF and showed that, on $NaDodSO_4$/polyacrylamide gels electrophoresed under reducing conditions, the HUT-102B2 cell receptor migrates as a M_r 50,000 glycoprotein (p50) (9). In contrast, the receptor on phytohemagglutinin (PHA)-activated normal peripheral blood T cells is larger (M_r higher by ≈ 5,000–8,000) than that on HUT-102B2 cells because of differences in post-translational processing (unpublished observations; ref. 11). HUT-102B2 cells were used in many of the studies reported herein because of their large number of receptors (6, 9), rapid growth rate, and clonality of the cell line. HUT-102B2 cells were derived from a lymph node of a patient with a mature T-cell lymphoma (12) and shed a type C retrovirus termed human T-cell leukemia/lymphoma virus (HTLV) (13), which is etiologically associated with adult T-cell leukemia (ATL) (14).

We now provide stronger evidence that anti-Tac recognizes the TCGF receptor and more completely characterize the receptor. We will always refer to the Tac antigen on HUT-102B2 cells as p50 (its size on reducing gels), even though on nonreducing gels it migrates with an M_r of ≈45,000.

MATERIALS AND METHODS

Competitive Binding Experiments. Peripheral blood mononuclear cells were activated with E-PHA (Burroughs Wellcome, Research Triangle Park, NC) at 0.5 μg/ml for 72 hr in RPMI 1640 medium containing 10% fetal bovine serum. Cells were washed and suspended at 2×10^{7} cells per ml in RPMI 1640 medium containing 25 mM Hepes (pH 7.4) and 1% bovine serum albumin; 1×10^{6} cells were incubated for 60 min with 0–500 ng of purified TCGF at 4°C in a final volume of 150 μl. Less than saturating doses of ^{3}H-labeled anti-Tac [10^{5} cpm; labeled as described by Tack *et al.* (15)] or ^{125}I-labeled anti-T11 (10^{6} cpm; iodinated with chloramine T) were added, and cells were incubated for another 60 min. Cells were washed, and bound and free radiolabeled ligand were separated by centrifuging cells through 1 M sucrose in a Microfuge.

Crosslinking Experiments. TCGF from PHA- and phorbol ester-induced JURKAT leukemic cells was purified by immunoaffinity chromatography (16) and migrated as a single spot on two-dimensional (2-D) gels. TCGF was crosslinked to HUT-102B2 cells with disuccinimidyl suberate (DSS) by the method of Kasuga *et al.* (17). Briefly, 1×10^{7} HUT-102B2 cells were incubated with 400 ng of purified TCGF at room temperature for 30 min, by which time saturation binding had occurred (6). The cells were then crosslinked in 1 ml of phosphate-buffered saline containing 0.1 mM DSS and 4% dimethyl sulfoxide at room temperature for 20 min or sham crosslinked by omitting the DSS. The reactions were quenched with 3 ml of 10 mM Tris·HCl/1 mM EDTA, pH 7.4, for 5 min. Cells were washed, surface-iodinated as described (9), and immunoprecipitated with anti-Tac, anti-TCGF, or control UPC10 (an IgG2a-κ, Litton Bionetics). Some crosslinking experiments were performed with cells biosynthetically labeled with [^{35}S]methionine (New England Nuclear, >1,000 Ci/mmol; 1 Ci = 37 GBq) prior to crosslinking. Immunoprecipitates were electrophoresed on nonreducing 8.75% $NaDodSO_4$/polyacrylamide gels.

Reducing vs. Nonreducing Conditions. Cells (1×10^{7}) were labeled overnight with 0.5 mCi of D-[^{3}H]GlcN (New England Nuclear, 32.5 Ci/mmol), immunoprecipitated with anti-Tac (1), and electrophoresed on 8.75% $NaDodSO_4$/polyacrylamide gels in the presence or absence of 0.1 M dithiothreitol.

Electrophoretic Transfer Blotting. p50 was partially purified by anti-Tac immunoprecipitation from HUT-102B2 cells, electrophoresed on $NaDodSO_4$ gels, and transferred to nitrocellulose in a Hoefer Transphor Electrophoresis unit by using 0.2 A for either 4 or 16 hr. Transfer was confirmed by staining of parallel transfers to nitrocellulose with amido black and of

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Abbreviations: TCGF, T-cell growth factor; DSS, disuccinimidyl suberate; PHA, phytohemagglutinin; 2-D, two dimensional.

the gels with Coomassie G250 (18). After transfer, the nitrocellulose was immunoblotted by using 75 μg of purified anti-Tac or control antibody (RPC5, Litton Bionetics), followed by 10 μCi of ^{125}I-labeled heterologous rabbit anti-mouse IgG (New England Nuclear, 2–10 μCi/μg) as described (19, 20).

2-D Gel Electrophoresis. These were performed as described by O'Farrell (21). The isoelectric focusing first dimension pH gradient was established by using Pharmalyte 3–10 Ampholines (Pharmacia). The second dimension was a reduced 7.5% NaDodSO$_4$/polyacrylamide gel.

Prevalence of p50 on HUT-102B2 Cells. Cells were labeled overnight with [^{35}S]methionine or [^{3}H]leucine and then solubilized in 1 ml of 10 mM Tris·HCl (pH 7.4) buffer containing 0.14 M NaCl, 1% Triton X-100, and 100 μg of phenylmethylsulfonyl fluoride per ml. Radioactivity incorporated into protein was determined by trichloroacetic acid precipitation of a 5-μl aliquot. The remaining extract was then immunoprecipitated with anti-Tac antibody and electrophoresed on a tube gel. The gel was sliced, and each slice was solubilized with 10 ml of 3% Protosol/4.2% Liquifluor (both from New England Nuclear) in toluene at 37°C for 4 hr. The percentage of *de novo* protein synthesis represented by p50 was estimated by the ratio of the specific radioactivity in the slices corresponding to p50 to the total precipitable radioactivity in the extract.

Pulse–Chase Labeling. HUT-102B2 cells (6 × 10^7) were washed and resuspended in methionine-free media; 0.4 mCi of [^{35}S]methionine (≈125 pmol) was added, and the cells were incubated at 37°C for 15 min. Excess unlabeled methionine (3 mmol) was then added, and culture was continued for 0, 15, 30, 60, 120, or 240 min, followed by extraction, immunoprecipitation with anti-Tac, and analysis by NaDodSO$_4$/polyacrylamide gel electrophoresis.

Studies with Tunicamycin, Neuraminidase, and Endoglycosidase F. Tunicamycin was dissolved at 2 mg/ml in 0.1 M NaOH, and aliquots were stored in liquid nitrogen. Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 0–5 μg of tunicamycin per ml for 16 hr at 37°C (22). Cells then were washed and incubated in methionine-free media containing the same concentrations of tunicamycin and 0.4 mCi of [^{35}S]methionine for 4 hr at 37°C. Cells then were pelleted, extracted, immunoprecipitated with anti-Tac or control UPC10, and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. Neuraminidase (Calbiochem) reactions were performed by treating immunoprecipitates with 0.1 unit of neuraminidase for 1 hr at 37°C in the presence of 10 mM CaCl$_2$. Endoglycosidase F reactions were as described by Elder and Alexander (23). Immunoprecipitates were boiled for 2 min in 100 mM sodium phosphate, pH 6.1/1% Nonidet P-40/0.1% NaDodSO$_4$/1% 2-mercaptoethanol/50 mM EDTA and then were treated with endoglycosidase F for 2 hr at 37°C.

RESULTS

TCGF Blocks the Binding of Anti-Tac to PHA-Activated Lymphoblasts. When present in large amounts, TCGF blocked 85% of ^{3}H-labeled anti-Tac binding to PHA-activated lymphoblasts but did not block the binding of ^{125}I-labeled anti-T11, which binds to the sheep erythrocyte receptor present on these cells (Fig. 1). These data support the contention that anti-Tac recognizes the TCGF receptor.

Crosslinking of TCGF to HUT-102B2 Cells. We next attempted to perform crosslinking experiments with radiolabeled TCGF. Our efforts to iodinate or tritiate purified TCGF to high specific activity resulted in loss of specific binding of TCGF for cells bearing TCGF receptors. After crosslinking with TCGF that had been labeled biosynthetically with [^{3}H]leucine and [^{3}H]lysine, we were unable to identify any band on gels even when exposed for >1 month with preflashed Kodak XAR-5 film, Dupont intensifying screens, and a gel enhancer. Therefore, we radiolabeled all cell surface proteins, including the putative TCGF receptor (p50), and attempted to identify a change in p50 after crosslinking.

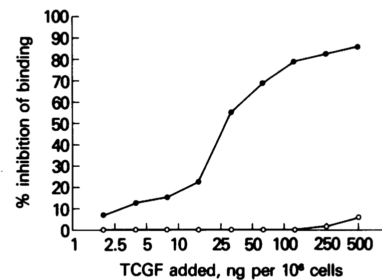

FIG. 1. TCGF blocks the binding of ^{3}H-anti-Tac to PHA-activated lymphoblasts. The amount of TCGF added is on the abscissa and the percentage inhibition of binding of ^{3}H-labeled anti-Tac (●) or ^{125}I-labeled anti-T11 (○) is on the ordinate.

Fig. 2 shows TCGF covalently crosslinked to HUT-102B2 cells with DSS (Fig. 2 *Left*, lanes 1, 2, and 3) and cells treated identically but not crosslinked (Fig. 2 *Left*, lanes 4, 5, and 6). Cells were surface iodinated and immunoprecipitated with anti-Tac, anti-TCGF, or control UPC10. The typical diffuse band (band A) immunoprecipitated by anti-Tac was identified (lane 1; see also Fig. 3*a*, lane A, for migration of this band under nonreducing conditions), but in addition a second band (band B) of higher M_r (by 12,000–14,000) was identified. Anti-TCGF (Fig. 2 *Left*, lane 2) also identified this new band, whereas UPC10 (lane 3) did not. In contrast, when cells were sham crosslinked, anti-Tac (lane 4) immunoprecipitated p50 (band A) but not the new band (band B), and neither anti-TCGF nor UPC10 immunoprecipitated either band. Fig. 2 *Right* shows a similar experiment with [^{35}S]methionine-labeled cells. Both experiments demonstrate the appearance of a new band after crosslinking but not after sham crosslinking of TCGF to its receptor. The band was immunoprecipitated by both anti-Tac and anti-TCGF and was of a size consistent with its representing TCGF cross-

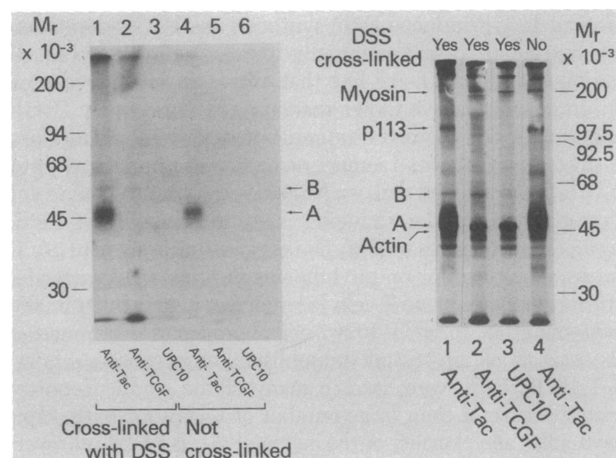

FIG. 2. Crosslinking experiments. (*Left*) Lanes: 1–3, HUT-102B2 cells crosslinked to TCGF with DSS, surface-iodinated, and then immunoprecipitated with anti-Tac (lane 1), anti-TCGF (lane 2), or UPC10 (lane 3); 4–6, same as lanes 1–3 except that DSS was omitted. Under the conditions used, crosslinking efficiency is low, perhaps explaining the relative lack of intensity of band B (putative covalently bound receptor–ligand complex). (*Right*) Lanes: 1–3, HUT-102B2 cells labeled with [^{35}S]methionine, crosslinked to TCGF with DSS, and immunoprecipitated with anti-Tac (lane 1), anti-TCGF (lane 2), or UPC10 (lane 3); 4, cells were sham crosslinked and immunoprecipitated with anti-Tac.

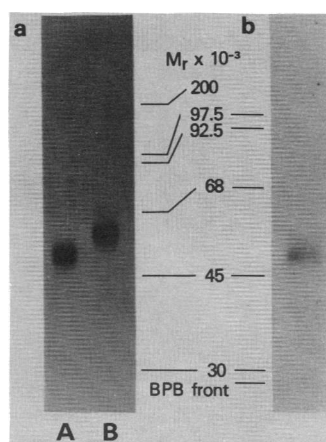

FIG. 3. (*a*) Tac antigen electrophoresed in the absence and presence of dithiothreitol. Anti-Tac immunoprecipitations of D-[^{3}H]GlcN-labeled HUT-102B2 cells were electrophoresed in the absence (lane A) or presence (lane B) of 0.1 M dithiothreitol on an 8.75% discontinuous NaDodSO$_4$/polyacrylamide gel. (*b*) Electrophoretic transfer blotting of p50 with anti-Tac. Blotting was on nitrocellulose with anti-Tac and ^{125}I-labeled rabbit anti-mouse IgG antibody. BPB, bromophenol blue.

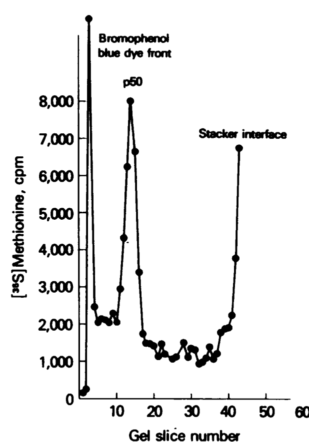

FIG. 5. p50 represents 0.05% of total HUT-102B2 *de novo* protein synthesis. Shown are the distribution of counts from an anti-Tac immunoprecipitation from [^{35}S]methionine-labeled HUT-102B2 cells when electrophoresed on a 7.5% NaDodSO$_4$/polyacrylamide tube gel. This experiment was performed under nonreducing conditions in which actin is polymerized with myosin as actomyosin; this accounts for the radioactivity at the stacker interface.

linked to its receptor. These data suggest that the TCGF receptor is recognized by anti-Tac.

Reducing vs. Nonreducing Conditions. Fig. 3*a* demonstrates the differential migration of Tac antigen in the absence or presence of dithiothreitol. Under reducing conditions (lane B), the precipitated protein had a M_r of ≈50,000 as reported (9). Under nonreducing conditions (lane A), the protein migrated more rapidly with an M_r of ≈45,000, consistent with the presence of intrachain disulfide bond(s).

Electrophoretic Transfer Blotting of p50 with Anti-Tac. This technique was used to help confirm that p50 is recognized directly by anti-Tac rather than being coimmunoprecipitated. Anti-Tac recognized p50 in immunoblots performed under nonreducing conditions (Fig. 3*b*). In data not shown, anti-Tac did not recognize p50 in an immunoblot electrophoresed under reducing conditions. Thus, intact intrachain disulfide bond(s) appear critical for anti-Tac binding to p50. When control RPC5 was used instead of anti-Tac, p50 was not identified, indicating that p50 is not being labeled fortuitously by the second agent (heterologous ^{125}I-labeled rabbit anti-mouse IgG). When performed on crude HUT-102B2 cellular extracts, electrophoretic transfer blotting did not identify any band, perhaps because of the need for larger amounts of and renaturation of the NaDodSO$_4$-denatured p50 in order to image it with anti-Tac. In accordance with this hypothesis, anti-Tac has markedly diminished ability to immunoprecipitate p50 when cellular extracts are boiled in 1% NaDodSO$_4$ prior to immunoprecipitation.

2-D Gel Electrophoresis of p50. Anti-Tac immunoprecipitations from [^{35}S]methionine-labeled HUT-102B2 cells was analyzed on a 2-D gel (Fig. 4). Consistent with its being a glycoprotein with intrinsic heterogeneity, p50 has a broad pI ranging from 5.5 to 6.0. Because cells were labeled with [^{35}S]methionine rather than D-[^{3}H]GlcN, actin was seen and migrated with a pI slightly more acidic than that reported for other eukaryotic actins (24). p113, a nonsurface protein previously reported as being coimmunoprecipitated with p50 (9), was also identified. The receptor on PHA-activated lymphoblasts had a slightly more acidic pI of 5.3–5.7 (data not shown).

Prevalence of p50 in HUT-102B2 Cells. Fig. 5 shows the distribution of radioactivity obtained when an anti-Tac immunoprecipitation of biosynthetically labeled HUT-102B2 cells was electrophoresed on a tube gel under nonreducing conditions. Whether [^{35}S]methionine (shown) or [^{3}H]leucine (not shown) was used as the labeling amino acid, and whether the tube gel was run under nonreducing (shown) or reducing (not shown) conditions, a similar result of 0.05% was obtained as the approximate prevalence of p50, calculated as described.

Pulse–Chase Labeling of HUT-102B2 Cells. [^{35}S]Methionine pulse–chase experiments were performed to evaluate the time course of p50 synthesis and to define whether precursor forms exist (Fig. 6). By 1 hr of chase, p50 was evident. p113, a protein noted previously (9) in anti-Tac immunoprecipitations of [^{35}S]methionine-labeled cells, was not labeled until after p50, indicating that p113 is not a precursor of p50. Because p50 is a glycoprotein (Fig. 3*a*; ref. 9), we hypothesized that a smaller precursor might exist. It is therefore of interest that two smaller proteins (p35 and p37) that were specifically immunoprecipitated with anti-Tac appeared prior to the labeling of p50, and both disappeared as p50 appeared.

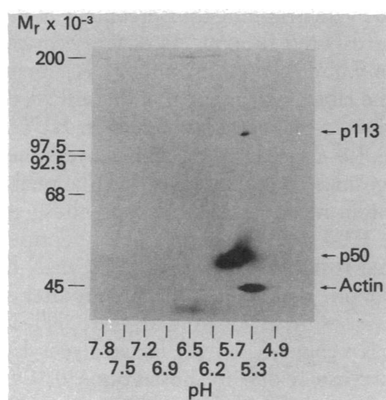

FIG. 4. 2-D gel of p50. The pH of the isoelectric focusing dimension is on the abscissa, and migration of molecular weight markers is on the ordinate. The spots corresponding to p113, p50, and actin are indicated.

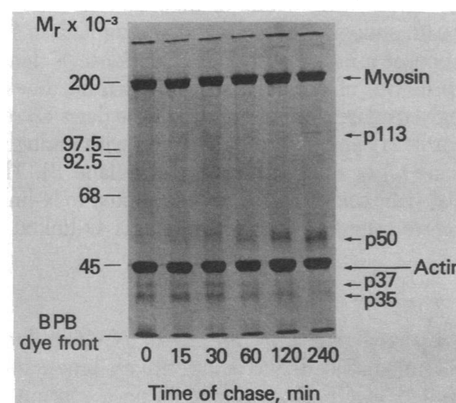

FIG. 6. Pulse–chase labeling of HUT-102B2 cells with [^{35}S]methionine. Cells were pulsed for 30 min with [^{35}S]methionine and then chased with excess unlabeled methionine for the time period indicated on the abscissa, followed by extraction and immunoprecipitation with anti-Tac. Locations of myosin, p113, p50, actin, p37, and p35 are indicated. BPB, bromophenol blue.

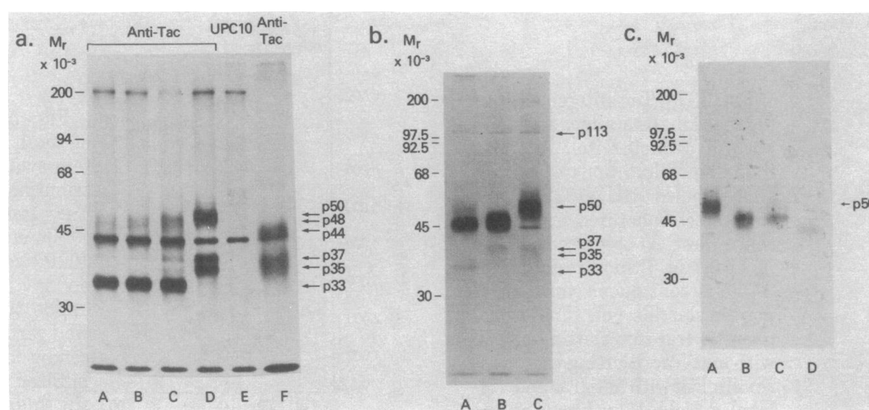

FIG. 7. (*a*) HUT-102B2 cells were labeled with [^{35}S]methionine in the presence of tunicamycin at 5 μg/ml (lane A), 2.5 μg/ml (lane B), or 1.25 μg/ml (lane C) or in the absence of tunicamycin (lanes D–F) and then were immunoprecipitated with anti-Tac (lanes A, B, C, D, and F) or control UPC10 (lane E). Lane F shows the results of pH 2.0 treatment for 30 min at 80°C of material identical to that in lane D. Location of p50, p48, p37, p35, and p33 are indicated. (*b* and *c*) Studies with neuraminidase and endoglycosidase F: anti-Tac immunoprecipitations from [^{35}S]methionine-labeled HUT-102B2 cells (*b*) not digested (lane C) or digested with neuraminidase (lane B) or endoglycosidase F (lane A); anti-Tac immunoprecipitations from D-[^{3}H]GlcN-labeled HUT-102B2 cells (*c*) not digested (lane A) or digested with neuraminidase (lane B), endoglycosidase F (lane C), or neuraminidase followed by endoglycosidase F (lane D).

Studies with Tunicamycin, Neuraminidase, and Endoglycosidase F. We next investigated N-linked glycosylation in p50 with tunicamycin, which inhibits such glycosylation by blocking the transfer of *N*-AcGlcN-1-phosphate from UDP-*N*-AcGlcN to dolicholmonophosphate (25, 26). As shown in Fig. 7*a*, cells were incubated with 0–5 μg of tunicamycin per ml and then labeled with [^{35}S]methionine for several hours. In this pulse-labeling experiment, in the absence of tunicamycin, anti-Tac (Fig. 7*a*, lane D) but not control UPC10 (lane E) immunoprecipitated p35, p37, and p50. When tunicamycin was added (lanes A, B, and C), p50 appeared to diminish in M_r by $\approx$2,000 (p48), and p35 and p37 disappeared and a M_r 33,000 band appeared. These transitions presumably represent blockade of N-linked glycosylation. The shift in size of p50 and absence of shift in p35 and p37 after mild acid hydrolysis (pH 2.0 for 30 min at 80°C with 0.01 M HCl) suggests the presence of sialic acid residues only in p50 (lane F). In Fig. 7*b*, changes in the size of [^{35}S]methionine-labeled p35, p37, and p50 (lane C) after digestion with neuraminidase (lane B) and endoglycosidase F (lane A) are shown. The decrease in size of p50 but not of p35 and p37 after neuraminidase treatment confirms that sialic acid is present only in p50. Digestion with endoglycosidase F, which cleaves N-linked sugars (23), recapitulated the tunicamycin experiment (Fig. 7*a*) and demonstrated that p50, p35, and p37 each contain N-linked sugars. In Fig. 7*c*, the results of digestion of D-[^{3}H]GlcN-labeled p50 with endoglycosidase F and neuraminidase are shown. Identification of a band after endoglycosidase F (lane C) suggests the presence of O-linked sugars. Further, digestion with both endoglycosidase F and neuraminidase (lane D) resulted in a smaller protein band than did digestion with endoglycosidase F alone (lane C) or neuraminidase alone (lane B). These data indicate that sialic acid residues are not limited to N-linked sugar and, therefore, suggest that p50 contains O-linked carbohydrate.

DISCUSSION

We have reported that anti-Tac blocks TCGF-induced DNA synthesis in human T cells and competitively blocks the binding of radiolabeled TCGF to its receptors on a continuous T cell line (9). These findings suggested that anti-Tac recognized the receptor for TCGF, which we characterized as a M_r 50,000 glycoprotein in HUT-102B2 cells. In the present report, we demonstrate that preincubation of PHA-activated lymphoblasts with excess TCGF blocks the binding of anti-Tac. Further, after cross-linking purified TCGF (M_r 14,800) to radiolabeled HUT-102B2 cells, anti-Tac immunoprecipitates an additional protein (the putative TCGF–receptor complex) larger (M_r higher by $\approx$ 12,000–14,000) than the native receptor recognized by anti-Tac. This protein is also immunoprecipitated by anti-TCGF but not by control UPC10. These data provide added support that anti-Tac identifies the human receptor for TCGF. In PHA-activated lymphoblasts, the TCGF receptor is larger (M_r higher by 5,000–8,000) than that on HUT-102B2 cells, apparently because of differences in post-translational processing (unpublished observations; ref. 11).

The receptors on HUT-102B2 cells and on PHA-activated normal T cells (unpublished data) appear to be larger (M_r higher by several thousand) on reducing gels than on nonreducing gels, suggesting the presence of intrachain disulfide bond(s), with unfolding of the protein when reduced. Such bond(s) also are suggested by our inability to image the receptor with anti-Tac when electrophoretic transfer blots are performed under reducing conditions. A 2-D gel demonstrates that the HUT-102B2 receptor has an isoelectric point of 5.5–6.0. This broad band suggests that the protein(s) defined by anti-Tac is heterogeneous. It is probably not composed of highly diverse peptide chains because sharp peaks are obtained when p50 biosynthetically labeled with [^{35}S]methionine and [^{3}H]leucine is subjected to automated Edman degradation on a Beckman sequenator (unpublished data). Instead, the heterogeneity is most likely due to differences in glycosylation.

We obtained a crude estimate of the prevalence of the mRNA encoding p50 by determining the percentage of *de novo* protein synthesis represented by p50. As described, we calculated a prevalence of 0.05% when using either [^{3}H]leucine or [^{35}S]methionine as the label, suggesting that the mRNA corresponding to p50 is of low-to-moderate frequency in HUT-102B2 cells.

We used pulse–chase labeling, tunicamycin, neuraminidase, and endoglycosidase F to study post-translational processing of this glycoprotein receptor. We interpret these studies to suggest that the HUT-102B2 receptor has a peptide backbone of M_r 33,000 that is initially glycosylated by an N-linked mechanism to a p35/p37 doublet. About 30 min after synthesis, the precursor increases by an apparent M_r of 13,000–15,000, at least part of which is accounted for by O-linked glycosylation. Whether all of this increase is due to the addition of O-linked carbohydrate is uncertain. This degree of presumed O-linked glycosylation has been noted for the low density lipoprotein receptor (27). Sulfation, phosphorylation, or covalent union with another peptide chain are potential modifications that could be

involved. The mature TCGF receptor contains sialic acid; however, the N-linked precursor forms (p35 and p37) do not.

Thus, we have extended our initial characterization of the protein recognized by anti-Tac (9) by providing information regarding the peptide and carbohydrate structure of this receptor. The crosslinking and competitive binding studies support our earlier suggestion that this protein is the human receptor for TCGF. However, formal proof will require purifying the native receptor and demonstrating that it can bind both TCGF and anti-Tac. We initially were surprised that nearly complete inhibition of ^{3}H-labeled anti-Tac binding to cells was achieved only when 100- to 1,000-fold more TCGF was added than predicted for receptor saturation (6). Further, original estimates of receptor number with [^{3}H]TCGF consistently indicated fewer receptors (1/10 to 1/200) than were measured with ^{3}H-labeled anti-Tac. However, preliminary data suggest the existence of large numbers of previously undetected low-affinity TCGF receptors (unpublished data). If confirmed, this could account for the earlier discrepancies in receptor number detected and could explain why so much TCGF was needed for the inhibition of anti-Tac binding. This explanation would require that anti-Tac recognizes both classes of receptors. It is possible, though unproven, that the sharp receptor–ligand band detected in the crosslinking studies represents TCGF covalently crosslinked to the high-affinity receptor.

The observation that TCGF blocks the binding of anti-Tac might appear to be inconsistent with the ability of anti-Tac to immunoprecipitate a putative TCGF–TCGF receptor crosslinked complex. It is possible, however, that anti-Tac and TCGF recognize different epitopes on the receptor and that anti-Tac is capable of binding to receptors occupied by TCGF albeit more weakly (e.g., because of allosteric effects). This is in accord with our observation that intact anti-Tac antibody does not have agonist activity for T-cell proliferation. Alternatively, if the receptor exists as a dimer or higher polymer complex of p50 chains held together by noncovalent bonds, anti-Tac could immunoprecipitate a complex by binding to a noncrosslinked p50 associated with a p50 that has been crosslinked to TCGF. On $NaDodSO_4$ gels, one would then obtain the pattern depicted in Fig. 2.

This putative TCGF receptor is similar in size to many other human T-cell surface glycoproteins. For example, T1 (28), T4 (29), T5 (29), and T11 (30) have M_rs between 55,000 and 76,000. In addition, the M_r of a transforming growth-factor receptor has been identified as 60,000 (31). However, many of the classical growth-factor receptors are much larger. For example, the insulin (32) and insulin-like growth factor I (17) receptors under nonreducing conditions migrate with apparent M_rs of >300,000, and after reduction the M_r of the binding subunit is 130,000. The M_r of the insulin-like growth factor II receptor is 260,000 (17), of the nerve growth factor receptor is 143,000 (33), of the platelet-derived growth factor receptor is 164,000 (34), and of the epidermal growth factor receptor is 175,000 (35). Some but not all of these consist of multiple subunits. In identifying p50 as the putative TCGF receptor, we do not exclude that there are other subunits that together with p50 comprise a total receptor of greater size. Indeed, in [^{35}S]methionine biosynthetic labeling of HUT-102B2 cells and subsequent immunoprecipitation (1), we identified two other principal proteins (p113 and p180) that appeared to coimmunoprecipitate with p50 and could be part of a receptor complex. Neither appears to label with surface iodination or glucosamine; thus, we hypothesize that if they are part of the receptor, they do not have external tyrosine residues and may not be glycoproteins. It is also possible that in the native state p50 may exist as a polymeric structure on the membrane; alternatively, the TCGF receptor on HUT-102B2 cells may be a single monomeric protein of M_r 50,000.

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