DIRECT DEMONSTRATION OF THE IDENTITY
OF T CELL GROWTH FACTOR BINDING
PROTEIN AND THE TAC ANTIGEN

BY RICHARD J. ROBB* AND WARNER C. GREENE$†

From the * Central Research and Development Department, E. I. du Pont de Nemours &
Company, Glenolden Laboratory, Glenolden, Pennsylvania 19036; and the $ Metabolism Branch,
National Cancer Institute, Bethesda, Maryland 20205

The proliferation of T lymphocytes requires a lymphokine, T cell growth
factor (TCGF) or interleukin 2, together with the ability of the cells to respond
to its signal. Several studies have shown that responsiveness to the growth factor
is acquired during antigen or lectin stimulation (1–4). In fact, the presence of a
cell surface receptor for the factor was implied by the ability of cells to absorb
TCGF bioactivity (4). Direct demonstration of a specific, high affinity receptor
was later made using pure, biosynthetically radiolabeled growth factor (5). As
expected, binding of labeled TCGF was largely restricted to antigen or lectin
activated T cells. Recently, Leonard and co-workers (6) demonstrated that a
murine monoclonal antibody, termed anti-Tac (7), was able to block the binding
of labeled TCGF to a receptor-positive, human T cell line, HUT102B2. The
antibody bound a major component of 47,000–53,000 mol wt (designated p50)
and minor components of 115,000 and 180,000 mol wt from detergent-solubi-
lized cells. Similar experiments on phytohemagglutinin (PHA)-activated normal
lymphocytes demonstrated that the antibody recognized a slightly larger mole-
cule of 52,000–57,000 mol wt (designated p55).† Although the data suggested
that one of these components contained the binding site for TCGF, it was
impossible to ascertain which one was responsible. Moreover, it remained possible
that the anti-Tac–reactive molecules were unrelated to the TCGF receptor and
merely associated with it on the cell surface. In this paper, we demonstrate that
the p55 molecule that is recognized by anti-Tac is itself able to bind directly to
TCGF. This molecule thus appears to represent the cellular TCGF receptor.

Materials and Methods

Cell Labeling. Human peripheral blood lymphocytes were isolated using Ficoll-Paque
(Pharmacia Fine Chemicals, Piscataway, NJ) and were cultured (1 × 10⁶ cells/ml initial
concentration) at 37°C in RPMI 1640 containing 10% fetal bovine serum and 1 µg/ml
PHA (HA-16; Burroughs Wellcome Co., Research Triangle Park, NC). After 3 d, blast
cells were isolated on a step gradient of Percoll (Pharmacia Fine Chemicals) (8). For
labeling with [³⁵S]methionine, the cells were resuspended at 3 × 10⁶ cells/ml in methionine-
free minimum essential medium (MEM) (Gibco Laboratories, Grand Island, NY) with 1%  

† Leonard, W. J., J. M. Depper, T. A. Waldmann, and W. C. Greene. Aberrant receptors for T
cell growth factor in human T cell leukemia/lymphoma virus-infected human T cells. Manuscript
submitted for publication.
fetal bovine serum, 1 U/ml immunoaffinity-purified TCGF, and 100 μCi/ml [35S]methionine (1244.5 Ci/mmol; New England Nuclear, Boston, MA). The cells were harvested after 8 h at 37°C. For labeling with [3H]glucosamine, the blast cells were resuspended at 2 x 10^6 cells/ml in RPMI 1640, 10% fetal bovine serum with 1 U/ml TCGF and 20 μCi/ml [3H]glucosamine (32.5 GCi/mmol; New England Nuclear). The cells were harvested after 16 h at 37°C.

Solubilization. Radiolabeled blast cells (100 x 10^6) were solubilized by resuspension in 1.0 ml of 10 mM Tris, pH 7.5, 0.5% Nonidet P-40 (NP-40; Sigma Chemical Co., St. Louis, MO), 5 mM MgCl₂, and 2 μM phenylmethylsulfonyl fluoride (Sigma Chemical Co.). After 30 min at 0°C, the mixture was centrifuged at 100,000 g for 60 min. The supernatant was stored at -70°C.

Cell Binding. The inhibition of cellular binding of biosynthetically labeled, immunoaffinity-purified TCGF (9) by anti-Tac was measured by incubating (20 min, 37°C) serial dilutions of the antibody with radiolabeled factor ([3H]leucine, lysine TCGF [5]) and human PHA-blast cells (72 h culture). The amount of TCGF added was chosen to give 90% of the maximum binding to high affinity receptors. The cells were added at the initiation of the incubation rather than being pretreated with antibody (6). After incubation, the cells were washed and the binding levels of TCGF measured as previously described (5).

T Cell Growth Factor. TCGF was obtained from supernatants of the human T cell line, Jurkat, after stimulation of the cells with PHA and phorbol myristic acetate (10). The factor was enriched to 99+% purity using an immunoaffinity column coupled with a murine monoclonal antibody (9).

Affinity Supports. Purified anti-Tac antibody was coupled to cyanogen bromide-activated Sepharose 4B (Sigma Chemical Co.) by mixing a solution of antibody (1 mg/ml in 0.1 M NaHCO₃, pH 8.2) with an equal volume of beads for 3 h at 4°C. The reaction was quenched by the addition of 0.1 vol 0.2 M glycine, 0.1 M NaHCO₃, pH 8.2. Purified TCGF was coupled to Affigel 10 (Bio-Rad Laboratories, Richmond, CA) by mixing a solution of factor (1 mg/ml in 0.1 M Hepes, pH 7.2) with an equal volume of beads for 3 h at 4°C followed by quenching with glycine. In both cases, coupling efficiency exceeded 95%.

Affinity Fractionation of Cellular Protein. Aliquots (50 μl) of detergent-soluble preparations from radiolabeled PHA-blast cells were first cleared of nonspecifically binding material by incubation (50 min at 20°C) with 10 μl of Sepharose or Affigel 10 coupled with 1 mg/ml control murine IgG (Sigma Chemical Co.). The supernatant was then incubated with anti-Tac (4 μl) or TCGF-coupled beads (10 μl) for 60 min at 20°C followed by extensive washing with 10 mM Tris, pH 7.5, 0.14 M NaCl. For sequential analysis, the supernatant of the first incubation was reacted with a second fresh sample of anti-Tac or TCGF-coupled beads. Bound protein was recovered by boiling the washed beads for 5 min in sodium dodecyl sulfate (SDS) sample buffer (100 μl).

Gel Electrophoresis. Labeled molecules were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (8.75% acrylamide) according to the method of Laemmli (11). Radioactivity was visualized by fluorography using Enhance (New England Nuclear).

Results and Discussion

As previously shown by Leonard et al. (6) using the HUT 102B2 cell line, the anti-Tac antibody was able to totally inhibit the high affinity binding (Kd~3–6 x 10^-12 M) of [3H]leucine, lysine TCGF to human PHA-blast cells (Fig. 1). When the antibody was added to the culture at the same time as the growth factor, an ~50-fold molar excess of antibody over TCGF was required to achieve 50% inhibition of binding. Since the high affinity binding was directly correlated with the proliferative response of the cells (5), anti-Tac appeared to recognize a component of the physiological receptor or an associated cell surface molecule.

To directly compare TCGF-binding and anti-Tac-reactive molecules, deter-
gents-soluble preparations were made of PHA-induced blast cells biosynthetically labeled with either [35S]methionine or [3H]glucosamine. The preparations were incubated with either TCGF-coupled Affigel or anti-Tac-coupled Sepharose followed by SDS-PAGE analysis of the bound molecules. As shown in Fig. 2, both TCGF and anti-Tac-coupled supports specifically bound molecules of ~58,000 mol wt from either [35S]methionine- or [3H]glucosamine-labeled cells. Consistent with the observations of Leonard et al. (6), the bands visualized by fluorography were somewhat broader than those of standard proteins. The diffuse nature of the bands may be attributable to the fact that the molecule is highly glycosylated (12). In addition to the band at 58,000 mol wt, a faint band at 115,000 mol wt was occasionally detected using both TCGF and anti-Tac affinity supports. The inconsistency of its appearance may reflect a weak interaction with the 58,000 mol wt molecule or poor solubilization under these conditions.

The SDS-PAGE results suggested that the molecules recognized by anti-Tac and those able to bind to TCGF were glycoproteins of the same size. To demonstrate that they were identical, a preparation of [3H]glucosamine-labeled material was subjected to sequential binding by TCGF-coupled Affigel and anti-Tac-coupled Sepharose. Virtually all the anti-Tac-reactive material was bound during the first incubation with anti-Tac-Sepharose (Table I). Subjection of the supernatant from the first incubation to reaction with a second aliquot of anti-Tac-Sepharose resulted in the binding of little additional radiolabel. Incubation of the supernatant from this second reaction with TCGF-coupled Affigel also failed to result in significant binding, indicating that TCGF-binding molecules were included within the set of molecules bound by anti-Tac. When the converse series of incubations were performed, very little radiolabel was bound by anti-Tac after two successive incubations of the [3H]glucosamine-labeled material with TCGF-coupled Affigel. Thus, the anti-Tac-reactive molecules were included within those directly binding to a TCGF affinity support. SDS-PAGE analysis of the molecules bound at each step confirmed that all bound radiolabel was present in the 58,000 mol wt molecule(s) (data not shown).
**TABLE I**

*Sequential Binding of Radiolabeled Cellular Molecules to TCGF and Anti-Tac Affinity Supports*

| Sample | First incubation | Second incubation | Third incubation |
|--------|------------------|-------------------|------------------|
|        | TCGF Affigel     | Anti-Tac Sepharose| TCGF Affigel     | Anti-Tac Sepharose|
|        | dpm              |                   | dpm              |                   |
| 1      | 15,200(79)       | 20,100(96)        | 580(3)           | 350(1)            |
| 2      | 20,100(96)       | 3,100(16)         | 900(5)           |                   |

*Two identical samples (8 x 10⁶ dpm) of NP-40-solubilized molecules from [³H]glucosamine-labeled PHA-blast cells were subjected to three sequential incubations with TCGF-coupled Affigel (10 µl) and anti-Tac-coupled Sepharose (4 µl) as indicated. The results are given as disintegrations per minute of [³H]radiolabel in the pellet. Values in parentheses represent the percentage of the total bound radiolabel for the three incubations. Control IgG-coupled Sepharose or Affigel bound <1% of the above values.*

These results demonstrate directly that the 58,000 mol wt Tac antigen on PHA-blast cells contains a binding site for TCGF. Furthermore, they indicate that under these solubilization conditions, only anti-Tac-reactive molecules have this capability. Inhibition of TCGF bioactivity (6) and radiolabeled TCGF binding by anti-Tac can thus readily be explained by binding of the antibody to the cellular receptor for the factor. Inhibition could occur either as a result of steric blockade of the factor’s binding site or inducement of a conformational change that would alter the binding affinity for TCGF.

The results also indicate that the number of Tac proteins and TCGF receptors on the surface of PHA-blasts are equal. In contrast, quantitative binding assays demonstrated that, on a molar basis, ~5–10 times more radiolabeled anti-Tac antibody than radiolabeled TCGF was bound by such cells (W. Leonard, R. Robb, and W. Greene, unpublished observations). This discrepancy could arise for several reasons. First, although the specific activity of the radiolabeled probes has been repeatedly verified, any systematic error in these values would directly affect quantitation of the binding site numbers. Second, the level of binding of anti-Tac could be overestimated due to interaction with Fc receptors. Since similar results were obtained with Fab fragments of the antibody, however, this explanation is not applicable. Third, the TCGF-binding assay could underestimate the number of receptors if a large portion were already occupied by factor. This possibility also appears unlikely, however, since washing the cells at pH 4, which has been shown to quantitatively elute bound radiolabeled TCGF (R. Robb, unpublished observations), failed to significantly increase the subsequent measurement of TCGF binding. Finally, the discrepancy could arise from a difference in the affinity of the receptors for TCGF, similar to that found recently for the receptor for epidermal growth factor (13). If a large portion of the Tac antigens have a low affinity for TCGF, they would have gone undetected in the original radiolabeled TCGF-binding assays (5), which were conducted at low TCGF concentrations. Such a possibility would explain why sequential incubations of radiolabeled cellular molecules with TCGF-coupled Affigel have been consistently more effective than incubations with a single large aliquot of beads. Also, it would provide one possible explanation for the observation (12) that a large molar excess of TCGF is required to completely block the binding of radiolabeled anti-Tac despite the fact that in the reciprocal experiment (Fig. 1), an excess of anti-Tac was required to block the high affinity binding of TCGF.
FIGURE 2. SDS-PAGE analysis (8.75% acrylamide with 2-mercaptoethanol) of radiolabeled molecules from PHA-blast cells that were bound by the TCGF and anti-Tac affinity supports. (A) [35S]methionine-labeled cells with control IgG-coupled Sepharose/Affigel; (B) [35S]methionine-labeled cells with anti-Tac-coupled Sepharose; (C) [35S]methionine-labeled cells with TCGF-coupled Affigel; (D) [3H]glucosamine-labeled cells with control IgG-coupled Sepharose/Affigel; (E) [3H]glucosamine-labeled cells with anti-Tac-coupled Sepharose; (F) [3H]glucosamine-labeled cells with TCGF-coupled Affigel. A similar band of slightly smaller size was observed in each case when 2-mercaptoethanol was excluded.

The results presented here indicate that the 58,000 mol wt Tac antigen is a cellular binding site for TCGF. This protein may constitute the intact receptor or it may merely represent the binding site component of a larger, noncovalently bonded molecular complex. Further characterization of the receptor should be aided by TCGF-coupled supports, such as that described here, since the receptor can be quantitatively recovered by low pH elution (R. Robb, unpublished observations). Moreover, in contrast to anti-Tac, which is limited to recognizing the human receptor and that of a few monkey species, TCGF-coupled supports should be applicable to receptor purification in all species that are responsive to human factor (i.e., murine, rat, monkey, and guinea pig).

Summary
Radiolabeled molecules from detergent-solubilized human T cell blasts were fractionated on affinity supports coupled with T cell growth factor (TCGF) and anti-Tac antibody. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis indicated that a glycoprotein of ~58,000 mol wt was bound in both cases. Sequential binding to the two affinity supports demonstrated that the molecules recognized in each instance were identical. Thus, the Tac antigen contains the cellular binding site for TCGF.

Note added in proof: Preliminary binding experiments using high concentrations of [3H]leucine, lysine TCGF with a low specific radioactivity indicate the existence of a sizeable pool of receptor sites with an affinity 2,000–10,000 times lower than that of the high affinity receptors measured in Fig. 1. Such sites may explain the numerical discrepancy between early TCGF binding experiments (5) and the binding of the anti-Tac antibody. The hypothesis that the anti-Tac
antibody apparently reacts with both classes of receptor would explain its effect on the physiological response (high affinity binding) and the high level of Tac antigen on the cell surface.

We wish to acknowledge the excellent technical assistance of L. Gehman.

Received for publication 28 June 1983.

References
1. Morgan, D. A., F. W. Ruscetti, and R. Gallo. 1976. Selective in vitro growth of T-lymphocytes from normal human bone marrows. Science (Wash. DC). 193:1007.
2. Gillis, S., and K. A. Smith. 1977. Long-term culture of tumor-specific cytotoxic T-cells. Nature (Lond.). 268:154.
3. Larsson, E.-L., and A. Coutinho. 1979. On the role of mitogenic lectins in T-cell triggering. Nature (Lond.). 280:239.
4. Bonnard, G. D., D. Yosaka, and D. Jacobson. 1979. Ligand-activated T-cell growth factor-induced proliferation: absorption of T-cell growth factor by activated T-cells. J. Immunol. 123:2704.
5. Robb, R. J., A. Munck, and K. A. Smith. 1981. T-cell growth factor receptors. Quantitation, specificity, and biological relevance. J. Exp. Med. 154:1455.
6. Leonard, W. J., J. M. Depper, T. Uchiyama, K. A. Smith, T. A. Waldmann, and W. C. Greene. 1982. A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor: partial characterization of the receptor. Nature (Lond.). 300:267.
7. Uchiyama, T., S. Broder, and T. A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. J. Immunol. 126:1393.
8. Kurnick, J. T., L. Östberg, M. Stegagno, A. K. Kimura, A. Örn, and O. Sjöberg. 1979. A rapid method for the separation of functional lymphoid cell populations of human and animal origin on PVP-silica (Percoll) density gradients. Scand. J. Immunol. 10:563.
9. Robb, R. J., R. Kutny, and V. Chowdhry. Purification and partial sequence analysis of human T cell growth factor. Proc. Natl. Acad. Sci. USA. In press.
10. Robb, R. J. 1982. Human T-cell growth factor: purification, biochemical characterization, and interaction with a cellular receptor. Immunobiology. 161:21.
11. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (Lond.). 227:680.
12. Leonard, W. J., J. M. Depper, R. J. Robb, T. A. Waldmann, and W. C. Greene. Characterization of the human receptor for T cell growth factor. Proc. Natl. Acad. Sci. USA. In press.
13. Kawamota, T., J. D. Sato, A. Le, J. Polikoff, G. H. Sato, and J. Mendelsohn. 1983. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc. Natl. Acad. Sci. USA. 80:1337.