T CELL GROWTH FACTOR RECEPTORS

Quantitation, Specificity, and Biological Relevance*

By RICHARD J. ROBB, ALLAN MUNCK, and KENDALL A. SMITH

From the Immunology Program, Norris Cotton Cancer Center, and the Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire 03755

Recent studies have shown that although antigens and lectins initiate the activation of T cells, the proliferation of these cells is dependent on a 15,000-dalton glycoprotein designated T cell growth factor (TCGF)[1] (1–9). Accordingly, two antigen-dependent events are essential for the realization of a competent T cell proliferative response: the production of TCGF (5, 7), and the acquisition of a state of specific responsiveness to TCGF on the part of antigen-activated cells (10–13). As a result of this concept it has been proposed that there is a unique signaling apparatus within the immune system designed to transmit exogenous antigenic stimuli to an endogenous, hormonelike control system involving lymphokines (14, 15).

A number of experimental findings suggest that TCGF has characteristics similar to polypeptide hormones that interact with target cells by means of specific receptors. The mitogenic effect of TCGF is strictly concentration dependent such that the rate and extent of T cell clonal expansion is determined by the concentration of TCGF available (5, 10, 14, 15). Moreover, the effect of TCGF appears specific for T cells. TCGF promotes proliferation only of antigen- or lectin-activated T cells and has no mitogenic effect on unstimulated lymphocytes, activated B cells, or cells of other lineages (10–15). In addition, TCGF activity is absorbed only by activated T cells (10–15). The fact that such absorption is time, temperature, and cell concentration dependent, together with the finding that both viable and glutaraldehyde-fixed cells absorb the activity, has led us (10, 14, 15) and others (11–13) to propose that TCGF interacts with activated T cells by means of specific membrane binding sites.

The definitive demonstration of TCGF-specific binding sites depends upon the development of a radiolabeled TCGF binding assay. In this report we describe such an assay using highly purified, internally radiolabeled TCGF, and show that only TCGF-responsive T cells express high affinity, TCGF-specific binding sites. Moreover, the characteristics of the binding, and the close correlation of the binding and

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; Con-A, concanavalin A; CSA, colony-stimulating activity; CTLL, cytolytic T lymphocyte line; DMEM, modified Dulbecco's essential medium; EGF, epidermal growth factor; FCS, fetal calf serum; FGF, fibroblast growth factor; HTLL, helper T lymphocyte line; IEF, isoelectric focusing; IFNα, leukocyte interferon, type 1; IFNγ, immune interferon, type 2; LAF, lymphocyte-activating factor; LPS, lipopolysaccharide; MSA, multiplication-stimulating activity; NGF, nerve growth factor; PHA, phytohemagglutinin; PMA, phorbol myristic acetate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCGF, T cell growth factor; TdR, thymidine.

biological response curves, support the notion that TCGF, in a manner similar to polypeptide hormones, exerts its effects upon activated T cells through a cellular receptor.

Materials and Methods

Cell Cultures. Murine TCGF-dependent cytolytic (subclone 15H) (16) and helper (clone 18-33-3) (8) T lymphocyte lines were maintained as previously described at population levels between 1×10^4 and 5×10^5 cells/ml (doubling time ~20 h) in Iscove's Modified Dulbecco's Minimum Essential Medium (Iscove's DMEM; 17) supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum (FCS, Sterile Systems, Inc., Logan, Utah), 50 U/ml penicillin G, 50 µg/ml gentamycin, and 1.0 U/ml human TCGF derived from JURKAT T leukemia cells and partially purified by gel filtration (see following sections). All were cultured in a humidified atmosphere of 5% CO_2 in air at 37°C.

All human and primate cell lines were maintained in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% FCS, 50 U/ml penicillin G, 50 µg/ml gentamycin, and 300 µg/ml fresh L-glutamine at population levels between 2×10^5 and 1×10^6 cells/ml.

Human phytohemagglutinin (PHA) blasts were prepared by culturing peripheral blood lymphocytes isolated on Ficoll-Hypaque with 1 µg/ml PHA (Wellcome Reagents Limited, Beckenham, Eng.) in supplemented RPMI 1640 medium for 72 h. Alloantigen-activated human blasts were prepared by co-cultivation of peripheral blood mononuclear cells from two individuals (1×10^6 cells/ml) in supplemented RPMI 1640 medium for 120 h. Murine (BALB/c, The Jackson Laboratory, Bar Harbor, Maine) splenocytes and thymocytes were activated with concanavalin A (Con-A, 2.5 µg/ml, Miles Yeda, Rehovath, Israel), *Escherichia coli* lipopolysaccharide (LPS, 20 µg/ml, Grand Island Biological Co.), and allogeneic splenocytes (C57BL/6, The Jackson Laboratory) by culturing cells (2.5×10^6 cells/ml) for 48 h (Con-A, LPS) or 120 h (mixed lymphocyte culture) in DMEM (Grand Island Biological Co.) supplemented with 10% FCS, 50 U/ml penicillin G, 50 µg/ml gentamycin, 2-mercaptoethanol (5×10^{-5} M), and 300 µg/ml L-glutamine. When necessary, the proportion of blast cells was enriched to 90–95% by discontinuous gradient centrifugation (18).

TCGF Assay. TCGF biological activity was determined as previously described (5) by the TCGF concentration-dependent stimulation of proliferation of a cloned murine cytotoxic T lymphocyte line (CTLL-2, subclone 15H) (16). CTLL proliferation, as indicated by [³H]-thymidine (TdR) incorporation, was determined during the last 4 h of a 24-h culture period in the presence of serial twofold dilutions of a standard TCGF preparation and the experimental sample. The dilutions yielding 50% of the maximum CTLL [³H]TdR incorporation were determined, and that of the sample divided by that of the standard to give the concentration of TCGF in units per milliliter. The standard TCGF preparation, which was arbitrarily assigned a value of 1 U/ml, routinely yielded 50% of the maximal incorporation at a dilution of 1:10. Phorbol myristic acetate (PMA) and PHA, which were used to induce TCGF production, had negligible direct or synergistic (with TCGF) effects in the assay.

Production and Purification of Internally Radiolabeled TCGF. Radiolabeled TCGF was prepared by stimulation of a high-producer clone (clone 6) of the human T leukemia cell line JURKAT (19, 20), in the presence of a medium containing various radioactive amino acids. Since the release of TCGF from JURKAT cells was found to gradually decline with their time in culture, fresh aliquots of cells were thawed for each labeling experiment. The cells were grown for 3–5 d in culture after which they were washed with serum- and methionine-free DMEM (MEM Select-Amine Kit, Grand Island Biological Co.). The cells were resuspended at 5×10^6 cells/ml in the same medium, to which was added 1.5 µg/ml PHA (Wellcome Reagents), 50 ng/ml PMA (Consolidated Midland Corp., Brewster, N. Y.), 10 µM unlabeled methionine, and 0.5 mCi/ml [³⁵S]methionine (1,100 Ci/mmol, Amersham Corp., Arlington Heights, Ill.). The cell supernate was harvested after incubation for 14–18 h at 37°C, after which 1 mg/ml bovine serum albumin (BSA, Fraction V, Sigma Chemical Co., St. Louis, Mo.) was added as a carrier. Under these conditions ~40 U/ml of TCGF was recovered, representing 40–50% of the optimal

production obtained in normal, serum-free DMEM. Exclusion of the unlabeled methionine resulted in extremely low levels of secreted TCGF.

Similar conditions were used for labeling with ^{3}H-amino acids, except that DMEM containing the normal methionine level, 40 μM unlabeled leucine, 65 μM unlabeled lysine, 0.5 mCi/ml [^{3}H]leucine (55 Ci/mmol, ICN, Irvine, Calif.) and 0.5 mCi/ml [^{3}H]lysine (45 Ci/mmol) was substituted. Approximately 50-60% of the optimal release of TCGF was obtained. Lower concentrations of total leucine and lysine resulted in a marked decrease in TCGF levels.

After stimulation of JURKAT cells in the presence of radioactive amino acids, the cell supernate (20 ml) was concentrated 15-fold by ultrafiltration using a YM-5 membrane (Amicon Corp., Lexington, Mass.). Concentrated material (>95% recovery of TCGF biological activity) was chromatographed at 4°C on a 1.6 × 95-cm column of Sephadex G100 Superfine (Pharmacia Fine Chemicals, Piscataway, N. J.) in 10 mM Tris, pH 7.5, 0.15 M NaCl, and 100 μg/ml BSA. The yield of TCGF activity was ~76%. The pool of TCGF activity from the G100 column was equilibrated on a YM-5 membrane with a $\frac{1}{160}$ dilution of Pharmolyte, pH 6.5-9 (Pharmacia Fine Chemicals), and further fractionated by isoelectric focusing (IEF). Focusing was performed at 4°C on a 6 × 180-mm cylindrical gel (7% acrylamide, $\frac{1}{16}$ dilution of Pharmolyte, pH 6.5-9) at 1,000 V for 12 h. The gel was cut into 2-mm slices and eluted (72-96 h) in 0.5 ml of 10 mM Tris, pH 7.5, 0.15 M NaCl, 2 mg/ml BSA. The biological activity from each slice was measured in the TCGF assay and 2.5 μl was counted by liquid scintillation. A parallel gel (no sample) was sliced and eluted with 1 ml distilled water (4°C) for determination of a representative pH gradient. Radiolabeled TCGF preparations from the IEF gel were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on slab gels (12% acrylamide) according to the methods of Ziegler et al. (21) and Laemmli (22) with identical results. The gels were analyzed by fluorography (23).

Unlabeled TCGF was similarly prepared by stimulation of JURKAT cells with PHA and PMA, and purified in the absence of exogenous protein carriers by a combination of gel filtration and IEF (9). Such preparations contained no detectable type 1 or type 2 interferon activity (Frank Ruscetti, personal communication), macrophage-granulocyte colony-stimulating activity (Frank Ruscetti, personal communication), lymphocyte-activating factor activity, B cell growth factor (Benjamin Sredni, personal communication), or PHA (as assessed by direct mitogenicity on mouse thymocytes). Unlabeled TCGF was also prepared from rat splenocytes by stimulation with Con A as previously described (24).

Radiolabeled TCGF Binding Assay. All cell types were incubated for 2 h at 37°C in 50 ml RPMI 1640, 25 mM HEPES, pH 7.2 and washed three times (50 ml) in order to remove any endogenous TCGF. A small increase in the level of detectable receptors was found for the TCGF-dependent murine CTLL-2, subclone 15H cells after such treatment. After washing, cells were immediately used for binding experiments or were fixed with glutaraldehyde before binding. Glutaraldehyde fixation was performed by suspending the cells in 2% glutaraldehyde, 0.14 M NaCl, and 0.05 M cacodylate buffer (pH 7.3) for 15 min at 3°C, followed by extension washing (5 times) to remove glutaraldehyde (14).

To determine the level of binding, serial dilutions of [^{35}S]methionine- or [^{3}H]leucine, lysine-TCGF were incubated at 37°C with 0.5-2 × 10^6 cells in a total volume of 200 μl of RPMI 1640 supplemented with 25 mM HEPES, pH 7.2, and 10 mg/ml BSA (RPMI 1640-BSA) using 1.5 ml Eppendorf micro test tubes (2236-411-1, Brinkmann Instruments, Westbury, N.Y.). The tubes were mechanically rotated about their longest axis (horizontal position) at 20 rpm in a 37°C water bath. After 20 or 40 min, 1 ml ice-cold RPMI 1640-BSA was added to each tube and the cells were pelleted at 9,000 g for 10 s in an Eppendorf model 5413 centrifuge. The supernate was removed and counted by liquid scintillation to determine the level of unbound radioactivity. The cells in the pellet were resuspended with 100 μl RPMI 1640-BSA at 0°C and centrifuged at 9,000 g for 90 s through a 200-μl layer of a mixture (25°C) of 84% silicone oil (550 fluid, Contour Chemical Co., North Reading, Mass.) and 16% paraffin oil (Fisher Scientific Co., Pittsburgh, Pa.) to remove the small amount of unbound TCGF remaining in the original pellet. The tips of the tubes (400 μl polyethylene, Bio-Rad Laboratories, Richmond, Calif.) containing the cell pellet were cut-off, placed in scintillation vials, and the pellets solubilized by addition of 100 μl 1% SDS followed by 3 ml Biofluor (New England Nuclear, Boston, Mass.). The level of nonsaturable binding was determined by adding 25 nM unlabeled JURKAT-

derived TCGF (purified by gel filtration or by gel filtration and IEF) to each assay tube before addition of the cells. The counting efficiencies for the various [^{35}S]methionine-TCGF preparations and the bound and unbound fractions after cell binding were similar (90–95%) and no adjustment was made in the final results.

The following growth factors and hormones were examined for competition with the binding of radiolabeled TCGF: murine epidermal growth factor (EGF), rat multiplication-stimulating activity (MSA), murine 2.5s nerve growth factor (NGF), and bovine fibroblast growth factor (FGF), all from Collaborative Research Inc., Waltham, Mass.; murine lymphocyte-activating factor (LAF or interleukin 1) prepared by LPS-stimulation of P388D$_1$ cells (25); purified human leukocyte interferon type 1 (IFN-α) (a gift from Dr. Kurt Berg, University of Aarhus, Denmark), human immune interferon type 2 (IFN-γ) (a gift from Dr. William Stewart, Memorial Sloan-Kettering Cancer Center, New York), purified macrophage-granulocyte colony-stimulating activity (CSA) (a gift from Dr. Frank Ruscetti, National Cancer Institute, Bethesda, Md.), human urinary erythropoietin (2nd international standard), and bovine-porcine insulin (Eli Lilly and Company, Indianapolis, Ind.).

Radiolabeled TCGF: Time-Courses of Association and of Dissociation/Degradation.   A time-course of the association of [^{35}S]methionine-TCGF with murine CTLL cells (2.5 × 10^6 cells/ml) was determined at 4° and 37°C using a saturating level of radiolabeled TCGF (250 pM) in the absence and presence of unlabeled TCGF (25 nM). At various times, aliquots (200 µl) were removed and centrifuged (9,000 *g* for 90 s) through a 200-µl layer of 84% silicone oil:16% paraffin oil. The tips containing the cell pellet were cut off and the samples treated as described for binding assay.

The dissociation or release of intact or degraded [^{35}S]methionine-TCGF from murine CTLL cells was measured in two ways. In the first approach, a near-saturating level of [^{35}S]methionine-TCGF (150 pM) was allowed to bind to the cells for 60 min at 37°C. At that time, the concentration of free [^{35}S]methionine-TCGF was reduced by a 100-fold dilution of the cell suspension into RPMI 1640-BSA and the culture maintained at 37°C. Aliquots of the cell suspension (containing 5 × 10^5 cells) were removed at various time intervals and centrifuged as described above through a layer of silicone oil:paraffin oil to separate cell-associated and unassociated [^{35}S]methionine label. In a second approach, a similar level of [^{35}S]methionine-TCGF was allowed to bind to the CTLL cells (2.5 × 10^6 cells/ml) for 45 min at 4°C. At that time, the cells were pelleted and washed once with 1 ml RPMI 1640-BSA at 0°C to remove unassociated TCGF. The cells were resuspended at 2.5 × 10^6 cells/ml in RPMI 1640-BSA containing 25 nM unlabeled TCGF and incubated at 37°C. At various times aliquots (200 µl) were removed and the cells pelleted as before by centrifugation through silicone oil:paraffin oil. An estimate of the proportion of degraded [^{35}S]methionine-TCGF was made by precipitation of protein (4 h at 0°C) from aliquots of the complete cell suspension, or of washed cells, by adjusting each to a final concentration of 10% TCA (with 1 mg/ml BSA serving as a carrier). The supernate (degraded [^{35}S]methionine-TCGF) and precipitate (undegraded [^{35}S]methionine-TCGF) were counted separately.

Biological and Binding Responses.   A comparison was made between the percentage of the maximum biological response (cell proliferation as measured by [^{3}H]TdR incorporation) and the percentage of saturable binding of [^{35}S]methionine-TCGF at various concentrations of free TCGF. The level of binding was determined as described for binding assay using murine CTLL cells (2.5 × 10^6 cells/ml). The level of biological activity was determined as in a standard TCGF assay. Murine CTLL cells (2 × 10^4 cells/ml) were incubated at 37°C with quadruplicate sets of twofold dilutions of unlabeled TCGF in DMEM/10% FCS (200 µl total volume). After 20 h, the supernate from each dilution were harvested, titered in a TCGF assay, and compared with a standard TCGF preparation (1 U/ml) to determine the level of remaining free TCGF. The original cells were pulsed with [^{3}H]TdR for 4 h at 37°C, and the level of radioactivity incorporated was used as a measure of their biological response to TCGF.

Results

Purification of Internally Radiolabeled TCGF.   TCGF released by the human T leukemia cell line JURKAT, after stimulation with PHA and PMA in the presence of

[35S]methionine, was concentrated and chromatographed on a column of Sephadex G100 Superfine (Fig. 1A). TCGF biological activity eluted in a position corresponding to a globular protein of 19–20,000 mol wt and was coincident with a small peak of [35S]methionine label. The G100 pool was further fractionated by IEF on a polyacrylamide gel using a gradient of pH 6.5–9. As shown in Fig. 1B, the major peak of TCGF activity coincided with a major peak of [35S]methionine label (slices 21–23, pI ~8.2). A secondary peak of activity (slice 38, pI ~7.9), which represented a sialylated form of TCGF (9), also coincided with a peak of [35S]methionine label. The yield of TCGF biological activity in the major IEF peak as compared to the original cell supernate was 40%. Quantitatively and qualitatively similar results were obtained for fractionation of [3H]leucine, lysine-TCGF.

When analyzed by one-dimensional SDS-PAGE, followed by fluorography, the material from the major IEF peak migrated as a single band of radiolabel at 14,800 mol wt under nonreducing conditions and 15,500 mol wt under reducing conditions (Fig. 2). A single component of the same size was found by slicing a similar gel and recovering biological activity (9) and by electrophoresis and conventional staining (Coomassie Brilliant Blue R250) of unlabeled JURKAT TCGF purified by the same means (Richard Robb, unpublished observations). The similarity between the size estimates from gel filtration and SDS-PAGE suggests that a molecule of TCGF is composed of a single polypeptide chain. Despite the relative uniformity of the size and charge characteristics of [35S]methionine-TCGF from the major IEF peak, however, the potential for microheterogeneity requires further examination.

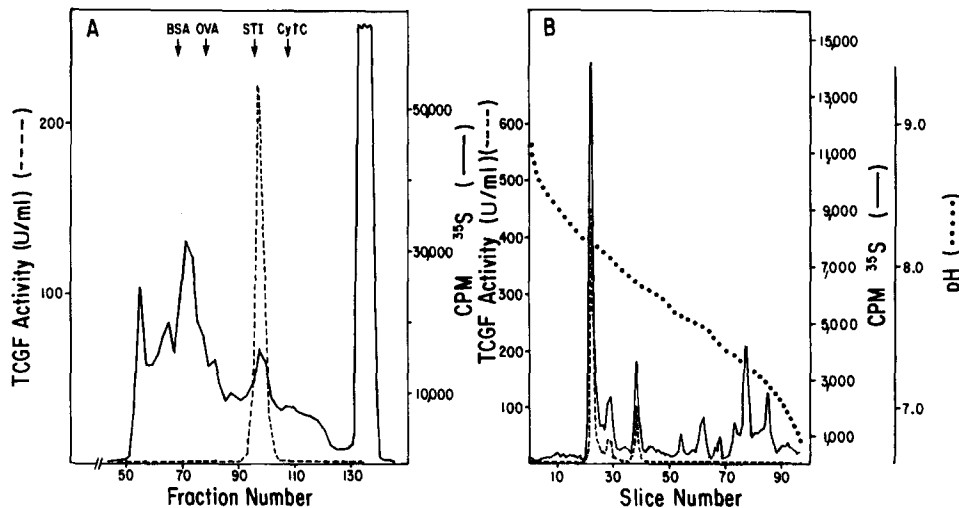The level of radioactivity in the major IEF peak depicted in Fig. 1B was 23,600

Fig. 1. (A) Elution profile of [35S]met-radiolabeled cell supernate derived from PHA and PMA-stimulated JURKAT cells on a 1.6 × 95-cm column of Sephadex G100 Superfine. A sample (4 μl) of every second fraction was used to determine the level of TCGF activity and 35S radiolabel. Standards used to separately calibrate the column were BSA, ovalbumin (OVA), soybean trypsin inhibitor (STI), and cytochrome c (CytC). (B) IEF (pH 6.5–9) profile of material from fractions 95–100 of the Sephadex G100 column. A sample of each fraction was tested for the presence of TCGF (5 μl) or counted to determine the level of 35S radiolabel (2.5 μl). The pH gradient was determined using a parallel gel (no sample).
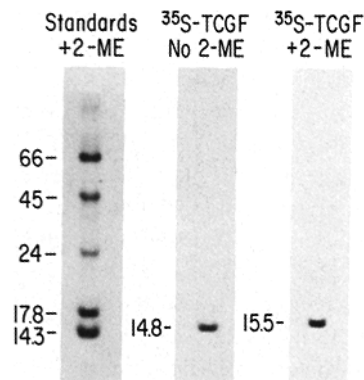
Fig. 2. SDS-PAGE analysis (with and without 2-mercaptoethanol [2-ME]) of [^{35}S]methionine-TCGF (4,000 cpm) eluted from slices 21–23 of the IEF gel (Fig. 1B). The radioactivity was visualized by fluorography. Exposure of the film for a 10-fold longer time did not reveal additional bands. The standards used in calibrating the gel were BSA (66,000), ovalbumin (45,000), trypsinogen 2(24,000), Mb (17,800), lysozyme (14,300), and were stained with Coomassie Brilliant Blue R-250.

cpm per unit of biological activity. By assaying the protein content of purified unlabeled JURKAT TCGF using amino acid analysis and a dye binding assay (26), it was determined that a unit of biological activity corresponds to ~8.2 ng of protein (Richard Robb, unpublished observations). Therefore, the specific activity of the [^{35}S]methionine-TCGF was estimated to be 2,880 cpm/ng or 44,600 cpm/pmol (mol wt = 15,500).

Time-Courses of Association and of Dissociation/Degradation of Radiolabeled TCGF. The optimal parameters for cell binding of radiolabeled TCGF were initially determined using a cloned murine, TCGF-dependent, T cytolytic line (CTLL-2, subclone 15H). Measurement of the time-course of association of [^{35}S]methionine-TCGF with these cells at 37°C revealed a very rapid uptake of radiolabel with maximum levels achieved within 15 min (Fig. 3A). The binding was saturable because in the presence of unlabeled TCGF (25 nM), very little [^{35}S]methionine-TCGF was associated with the cells at any time point. When the incubation was conducted at 4°C instead of 37°C, nearly the same maximum level of binding was reached, but only after 60 min of culture (Fig. 3A). Similar kinetics of association were found for human PHA blast cells.

Upon a 100-fold dilution of the CTLL cell suspension, most of the cell-associated ^{35}S-radiolabel (present after 1 h binding at 37°C) left the cells within 2 h at 37°C (data not shown). Analysis of the released radiolabel, however, revealed that a large portion (>70%) was no longer precipitable with 10% TCA in contrast to the nearly quantitative precipitation (>97%) of the original preparation of [^{35}S]methionine-TCGF. Thus, the radiolabeled TCGF appeared to be undergoing degradation. That such degradation might depend upon receptor binding was indicated by the finding that unlabeled TCGF (25 nM) added simultaneously with the [^{35}S]methionine-TCGF resulted in an absence of cell-associated radiolabel and a complete lack of degradation.

Measurement of the rate of conversion of cell-associated [^{35}S]methionine-TCGF into a TCA-soluble form at 37°C revealed a half-time of 60–70 min (data not shown). A similar value was obtained with [^{3}H]leucine, lysine-TCGF. Since the large number of leucines and lysines (≥30) in JURKAT-derived TCGF (Richard Robb, unpub-
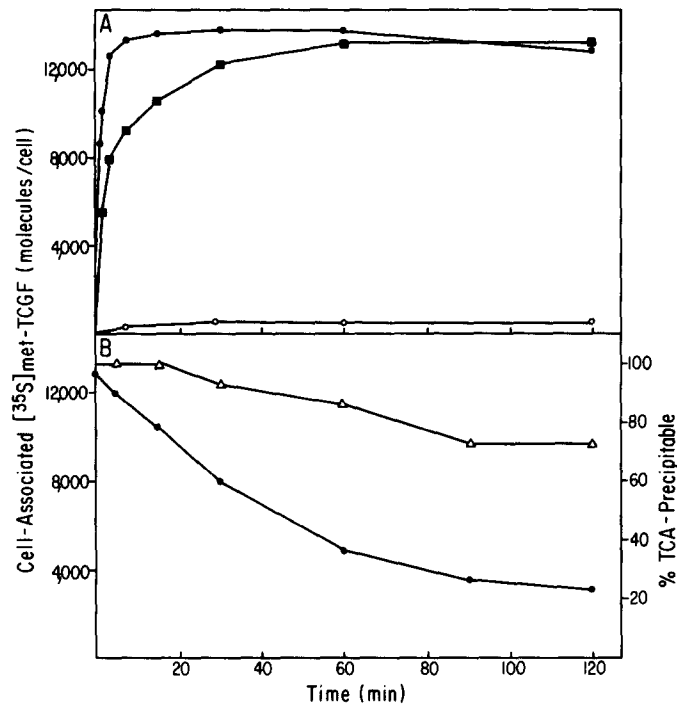
FIG. 3. (A) Time-course of the association of [^{35}S]methionine-TCGF with CTLL-2, subclone 15H cells in the absence (●, 37°C; ■, 4°C) and presence (○, 37°C) of 25 nM unlabeled TCGF. These and subsequent values for the number of TCGF binding sites per cell are based on the estimated specific activity of 44,600 cpm/pmol [^{35}S]methionine-TCGF. As an example, a level of 14,000 molecules per cell represents the binding of 520 cpm of [^{35}S]methionine-TCGF per 5 × 10^5 CTLL cells. (B) Time-course of the release (●) at 37°C of [^{35}S]radiolabel from CTLL cells following 45 min binding at 4°C, removal of unassociated radiolabel, and addition of 25 nM unlabeled TCGF to promote exchange. Aliquots of the whole cell suspension were removed at various times and adjusted to 10% TCA to determine the level of precipitable ^{35}S radiolabel (△).

lished observations) are likely to be distributed throughout the molecule, the conversion of the ^{3}H radiolabel to a TCA-soluble form implies general degradation of the molecule rather than removal of a small, methionine-rich section. In contrast to these results at 37°C, at 4°C virtually all of the radiolabel remained TCA precipitable for >2 h. Furthermore, inclusion of either ammonium chloride (10 mM) or chloroquine (100 μM), which have been shown to inhibit lysosome-dependent hormone degradation (27–32), resulted in >90% inhibition of the converstion of [^{35}S]methionine-TCGF to a TCA-soluble form at 37°C, while having no effect on the level of binding. Thus, degradation of TCGF is temperature dependent and appears to involve internalization and lysosomal association.

When binding was carried out for 45 min at 4°C to block degradation, followed by warming to 37°C and addition of 25 nM unlabeled TCGF to promote exchange, about half the cell-associated label was released within 45 min (Fig. 3B). Under these conditions within only about one-third of the [^{35}S]methionine-TCGF degraded, presumably because dissociation of labeled TCGF occurred faster than degradation. Thus, association of TCGF with CTLL cells is largely reversible and is characterized by rapid binding, slow dissociation ($t_{1/2}$ at 37°C of 40–50 min), and receptor- and

lysosome-dependent degradation of the molecule. Despite the degradation, the level of [^{35}S]methionine-TCGF associated with the cells (surface-bound and/or internalized, intact and/or degraded) remained stable for at least 60 min at 37°C (Fig. 3A). Subsequent measurements of binding capacity and receptor affinity were made after 20 or 40 min of culture at 37°C with little difference in the results.

Radiolabeled TCGF Binding: Cell Type, Number of Receptors, and Affinity. Incubation of increasing concentrations of [^{35}S]methionine-TCGF with antigen-specific, TCGF-dependent murine CTLL-2 (subclone 15H) and HTLL (clone 18-33-3) cell lines demonstrated that the binding consisted predominantly of a saturable component (Fig. 4A). Nonsaturable binding was estimated by including 25 nM unlabeled TCGF during the incubation. The level of binding that remained was linearly dependent upon the concentration of free [^{35}S]methionine-TCGF and generally constituted <4% of the total bound fraction in the absence of unlabeled TCGF. The average number of receptor sites per cell was ~15,000 for the CTLL and 13,000 for the HTLL (Table I). The sialylated form of [^{35}S]methionine-JURKAT TCGF (IEF slice 38, Fig. 1B) and the major IEF peak of [^{3}H]leucine, lysine-JURKAT TCGF gave similar estimates of the number of receptor sites for the CTLL line. In addition, glutaraldehyde-fixed CTLL cells bound almost as much [^{35}S]methionine-TCGF as viable cells (Table I).

Binding of [^{35}S]methionine-TCGF to murine splenocytes and thymocytes (BALB/c) indicated fewer than the minimum detectable level of 60 binding sites per cell (Fig. 4A, Table I). Upon stimulation with Con A or by alloantigen, however, a significant level of binding sites was found on both splenocyte and thymocyte blasts (Fig. 4A, Table I). In contrast, splenocytes stimulated with lipopolysaccharide were found to have no detectable receptors. These results are consistent with those of previous experiments in which only activated T cells, but not unstimulated lymphocytes or B cells blasts, absorbed TCGF biological activity (10–15). The fact that antigen-activated and lectin-activated cells both have binding sites with similar affinities for TCGF indicates that the activation step is the critical event in acquisition of TCGF responsiveness and that the lectin is not directly involved in the binding.

A Scatchard plot of the binding of [^{35}S]methionine-TCGF to the murine CTLL and HTLL cell lines and Con A-activated splenocytes indicated a linear relationship (Fig. 4B) and a similar apparent dissociation constant (Table I) in each case. These results imply that a single set of high-affinity binding sites is responsible for the saturable binding.

A similar analysis of binding to unstimulated human peripheral blood cells (90% lymphocytes) demonstrated an average of 200 binding sites per cell (Table I). Upon activation of the cells for 3 d with PHA or in a mixed lymphocyte reaction, the level of binding sites increased markedly to ~11,000 and 4,000 per cell, respectively (Fig. 5A, Table I). As with the murine cells, a linear relationship was obtained on the Scatchard plot (Fig. 5B). The apparent affinity of the human cell-surface receptor, however, was three to fourfold higher than that of the murine receptor (Table I).

A number of established human cell lines (including 10 T cell lines, 4 B cell lines, 2 myeloid cell lines, and a monocytoid cell line), which previous studies had shown to be unresponsive to TCGF (33), were found to have fewer than the detectable number of binding sites for [^{35}S]methionine-TCGF (Table II). In contrast, two T cell lines (HUT-102B2 and MLA-144) known to produce and respond to TCGF[2] (34, 35),
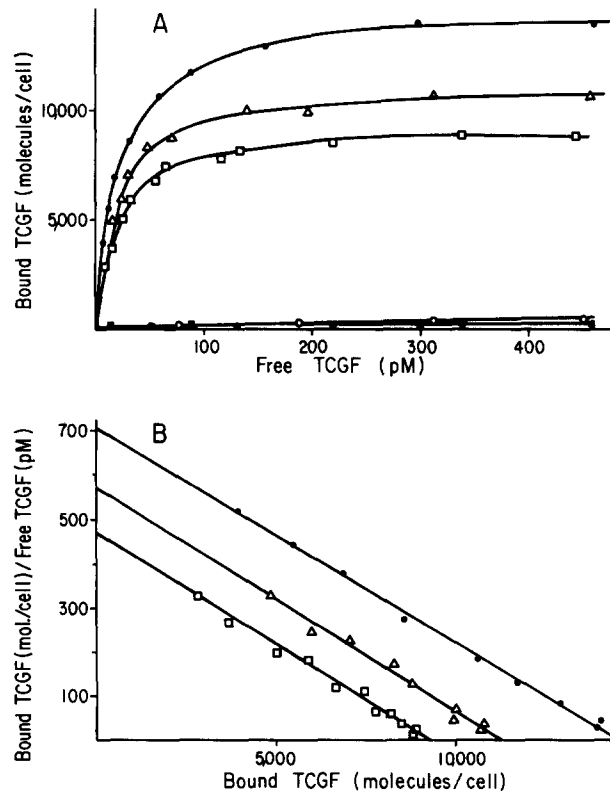
Fig. 4. (A) Binding of [^{35}S]methionine-TCGF to normal (■) and Con A-activated (□) murine splenocytes; the murine HTLL, clone 18-33-3 T helper cell line (△); and the murine CTLL-2, subclone 15H T cytolytic cell line in the absence (●) and presence (○) of 25 nM unlabeled TCGF. (B) A Scatchard plot of the data in A after correcting for nonsaturable binding.

expressed binding sites that had apparent affinity constants similar to that of human PHA blast cells (Fig. 5, Table II).

Competition for Radiolabeled TCGF Binding.   The specificity of radiolabeled TCGF binding for murine CTLL cells and human PHA blast cells was examined by introducing a variety of growth factors and hormones as potential competitors for the binding. In cases where physiological concentrations were known (i.e., EGF, NGF, insulin, interferon, erythropoietin, LAF), the factors were used at levels above those known to illicit a maximum response. As shown in Table III, none of the growth factors tested had any measurable effect on the binding of [^{35}S]methionine-TCGF to murine CTLL cells. Of special note is the finding that LAF, which provides a necessary signal for TCGF release by T cells (36–38), did not compete for [^{35}S]-methionine-TCGF binding. Moreover, human interferon, macrophage-granulocyte CSA, insulin, and erythropoietin also failed to exert a significant effect on [^{35}S]-methionine-TCGF binding to human PHA blast cells.

Competition for [^{35}S]methionine-TCGF binding was also examined using different preparations of TCGF. As shown in Table III, unlabeled TCGF derived from JURKAT cells (purified through IEF and/or gel filtration) almost completely abrogated [^{35}S]methionine-TCGF binding to both murine CTLL cells and human PHA

TABLE I

Characteristics of [³⁵S]Methionine-TCGF Binding to Unstimulated and Stimulated Cells

Cell type	Stimulant	[³⁵S]Methionine-TCGF binding*	
		Sites/cell	Dissociation constant $(\times 10^{-12} M)$
Murine cells			
Splenocytes	—	<60	—
Thymocytes	—	<60	—
Splenocytes	Con A	9,000 ± 340	19.6 ± 1.1
Splenocytes	Alloantigen	5,800 ± 400	24.6 ± 4.1
Thymocytes	Con A	3,180 ± 310	19.1 ± 1.5
Splenocytes	LPS	<60	—
CTLL-2, subclone 15H	—	14,800 ± 1,900	20.4 ± 0.8
CTLL-2, subclone 15H (glutaraldehyde-fixed)	—	13,200	—
HTLL, clone 18-33-3	—	12,700 ± 2,300	20.1 ± 1.6
Human cells			
Peripheral blood mononuclear cells	—	210	7.4
Peripheral blood mononuclear cells	PHA	10,900 ± 240	5.4 ± 1.1
Peripheral blood mononuclear cells	Alloantigen	4,300 ± 340	4.9 ± 0.4

* The calculated values of the number of binding sites per cell and the dissociation constant are based on the estimated specific activity of 44,600 cpm/pmol [³⁵S]methionine-TCGF and represent the mean and SEM of two or more determinations.

blast cells. Although JURKAT-derived TCGF migrates as a single major peak on IEF (pI 8.2), TCGF derived from normal human tonsil cells is heterogeneous with respect to charge, separating into three distinct forms (pI 8.2, 7.9, and 7.6) (9). Previous studies revealed that this heterogeneity is due to variable sialylation and glycosylation, and that the carbohydrate component is not essential for biological activity (9). It was of interest, therefore, to determine the relative competitive efficiency of each of the three species of tonsil-derived TCGF isolated from IEF gels in the [³⁵S]methionine-TCGF binding assay. When present at 2 nM TCGF, each of the three IEF fractions was capable of almost completely inhibiting [³⁵S]methionine-JURKAT TCGF binding to CTLL cells (Table III). Furthermore, when compared on the basis of biological activity (TCGF cell-proliferation assay), very similar concentrations of unlabeled JURKAT TCGF and of the three tonsil-derived IEF fractions were needed to reduce the binding of [³⁵S]methionine-TCGF by 50% (data not shown). The finding that tonsil-derived material of three distinct isoelectric points competed for [³⁵S]methionine-TCGF binding relative to its respective TCGF biological activity supports the impression that the substance responsible for the competition, as well as the binding, is TCGF, and not a contaminant in the JURKAT-derived preparations (both radiolabeled and unlabeled).

The concentration dependence of the competition of unlabeled TCGF with [³⁵S]-methionine-TCGF binding is shown in Fig. 6. Unlabeled JURKAT-derived TCGF competed for [³⁵S]methionine-TCGF binding to both murine CTLL cells and human PHA blast cells with nearly superimposable curves. The concentration of unlabeled TCGF leading to 50% competition was 0.068 U/0.2 ml, nearly the same as the concentration of [³⁵S]methionine-TCGF (0.066 U/0.2 ml) used in the reaction mixture. Since at this level of radiolabeled TCGF (175 pM) the binding was at saturation
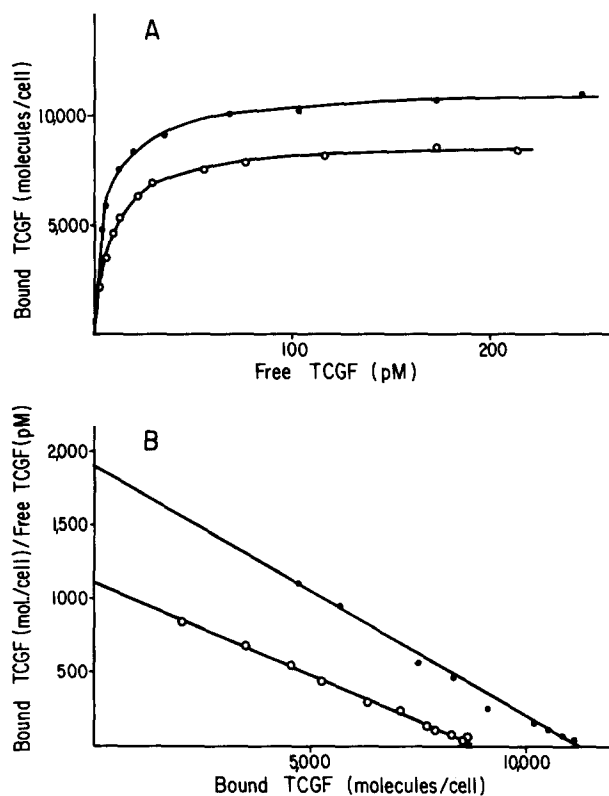
Fig. 5.   (A) Binding of [^{35}S]methionine-TCGF to human PHA blasts (●) and the MLA-144 cell line (○). (B) A Scatchard plot of the data in A after correcting for nonsaturable binding.

(Figs. 4A and 5A), addition of an equal amount of unlabeled TCGF would be expected to give ~50% competition.

In comparison to unlabeled human TCGF, TCGF derived from Con A-activated rat splenocytes was approximately six-fold less effective in competing with [^{35}S]-methionine-TCGF for binding to murine CTLL cells (50% competition occurred at 0.4 U/0.2 ml) (Fig. 6). This result could mean that human TCGF has a higher affinity for the murine cell binding site than does rat TCGF. Of particular note with regard to the correlation of [^{35}S]methionine-TCGF binding and biological activity was the finding that although rat TCGF competed for [^{35}S]methionine-TCGF binding to murine cells, there was no competition by rat TCGF for binding to human PHA blast cells (Fig. 6). This result corresponds to our prior findings on the species restriction of TCGF; i.e., that rat TCGF has mitogenic activity for murine cells but not for human cells (5).

Comparison of TCGF Biological Activity and Radiolabeled TCGF Binding.   In assessing the biological relevance of the binding demonstrated for radiolabeled TCGF, it was of particular interest to compare the concentrations of TCGF that result in significant binding with those responsible for in vitro proliferation. Serial two-fold dilutions of unlabeled JURKAT TCGF were incubated with the murine CTLL line as in a standard TCGF assay. After 20 h of culture, the supernates were harvested and placed

TABLE II

Characteristics of [³⁵S]Methionine-TCGF Binding of Cell Lines

Cell line	Cell type	[³⁵S]Methionine-TCGF binding*	
		Sites/cell	Dissociation constant ($\times 10^{-12}$ M)
CCRF-CEM	T	<60	—
HPB-MLT	T	<60	—
HSB-2	T	<60	—
JM	T	<60	—
JURKAT (clone 6)	T	<60	—
MOLT-3	T	<60	—
MOLT-4	T	<60	—
PEER	T	<60	—
RPMI 8402	T	<60	—
T-ALL-1	T	<60	—
HUT-102B2	T	13,000 ± 1,900	6.0 ± 0.6
DAUDI	B	<60	—
RM-1	B	<60	—
RPMI 1788	B	<60	—
RAJI	B	<60	—
K-562	Myeloid	<60	—
HL-60	Promyelocyte	<60	—
U-937	Monocytoid	<60	—
MLA-144 (clone 15)	Primate T cell	8,200 ± 2,100	8.0 ± 0.2

* Values represent the mean and SEM of two or more determinations.

into a second TCGF assay to measure the concentration of TCGF remaining after exposure to the cells. The cells from which the supernates were taken were then pulsed with [³H]TdR to measure their response to the original exposure to TCGF. When the data resulting from the [³H]TdR incorporation of the original cells were compared to the concentration of free TCGF remaining after the incubation, it was clear that, over a wide range, the rate of CTLL [³H]TdR incorporation was logarithmically related to the concentration of free TCGF. When the data from this experiment were compared to the binding curve for [³⁵S]methionine-TCGF to CTLL cells (Fig. 7), the concentration of TCGF that stimulated a given fraction of the maximum CTLL proliferation was remarkably similar to the concentration that resulted in the same fraction of occupied receptor sites. For example, the concentration of TCGF that promoted half-maximal [³H]TdR incorporation was 0.03 U/ml (16 pM) and the concentration of [³⁵S]methionine-TCGF giving half-maximal binding was 0.04 U/ml (21 pM). Moreover, the maximum biological response occurred at a concentration of TCGF that corresponded to ~90% saturation of the binding sites. The similarity between the biological dose-response curve and the [³⁵S]methionine-TCGF binding curve indicates that the biological response is nearly proportional to the TCGF-binding site occupancy.

Discussion

The restriction of radiolabeled TCGF binding to TCGF-responsive cells, the lack of competition by other growth factors and hormones, the high affinity of the binding,

TABLE III

Competition for [³⁵S]Methionine-TCGF Binding*

Potential competitors	Concentration	Target cells			
		Murine CTLL cells		Human PHA blast cells	
		sites/cell	% inhibition	sites/cell	% inhibition
Control	—	14,000 ± 340	0	10,300 ± 340	0
EGF	2 μM	14,000 ± 1,100	0		
NGF (2.5 s)	0.5 μM	14,100 ± 340	0		
MSA	1 μM	14,000 ± 200	0		
FGF	1 μM	13,600 ± 60	3		
LAF	60 U/ml	14,000 ± 340	0		
IFN-α	40,000 U/ml			10,000 ± 760	3
IFN-γ	40,000 U/ml			10,800 ± 60	0
CSA	—			10,100 ± 410	2
Erythropoietin	5 U/ml			10,400 ± 680	0
Insulin (bovine-porcine)	10 U/ml			10,200 ± 800	1
TCGF (JURKAT)	2 nM	150 ± 80	99	80 ± 20	99
TCGF (tonsil, IEF pI 8.2)	2 nM	110 ± 30	99		
TCGF (tonsil, IEF pI 7.9)	2 nM	140 ± 20	99		
TCGF (tonsil, IEF pI 7.6)	2 nM	180 ± 70	99		

* The assays were performed with [³⁵S]methionine-TCGF at a near-saturating concentration (~120 pM free TCGF for the murine CTLL and 75 pM free TCGF for the human PHA blasts). Values represent the mean and SEM of two determinations.

and the close correlation between the concentrations of TCGF that bind to cells and those that mediate the T cell proliferative response, all support the conclusion that the binding site detected in these studies is on the receptor through which the biological effects of TCGF are initiated. The implication of these findings is that, for the first time, it will be possible to apply a quantitative approach to physiological and pathophysiological studies of an immunologically important lymphokine and its receptor.

The lack of detectable TCGF binding sites on unstimulated murine lymphocytes, as well the presence of very few binding sites on unstimulated human peripheral blood lymphocytes, stands in contrast to the easily detectable levels found on alloantigen- and lectin-activated murine and human cells. These results provide an explanation for the observation that highly purified, lectin-free TCGF does not promote detectable mitosis of unstimulated lymphocytes, whereas TCGF initiates and maintains the proliferation of T cells once activated by antigen or lectin (10-15). The low level of binding sites found on unstimulated human peripheral blood lymphocytes (200 sites/cell) is just above the detection limit of the assay and represents only 30 cpm bound/2 × 10⁶ cells. Since the assay yields only an average level of binding in the whole cell population, the value of 200 sites/cell could represent a low level of receptor sites on most or all of the cells, or a high level of sites on a small fraction (<2%) of activated cells within the population. If this latter possibility were true, then the determination of the level of TCGF receptor sites in various disease states could yield information as to the proportion of circulating, activated T cells.

The increase in the level of receptor sites found upon alloantigen and lectin stimulation (>50-200-fold) mirrors earlier studies by ourselves and others where
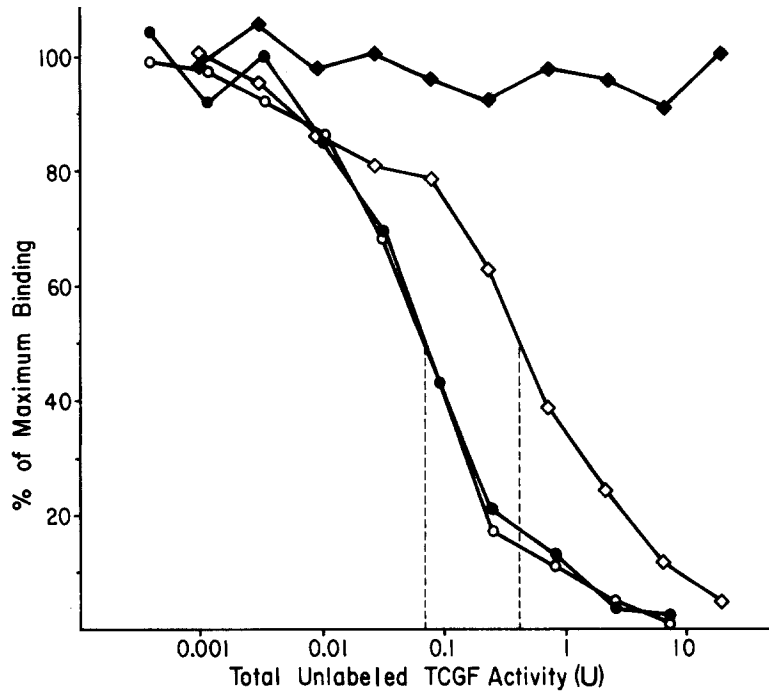
Fig. 6. Competition for the binding of [35S]methionine-TCGF (JURKAT) by unlabeled JUR-KAT-TCGF to human PHA blasts (●) and murine CTLL-2, subclone 15H cells (○), and by unlabeled Con-A-activated rat spleen-TCGF to human PHA blasts (■) and murine CTLL-2, subclone 15H cells (□).
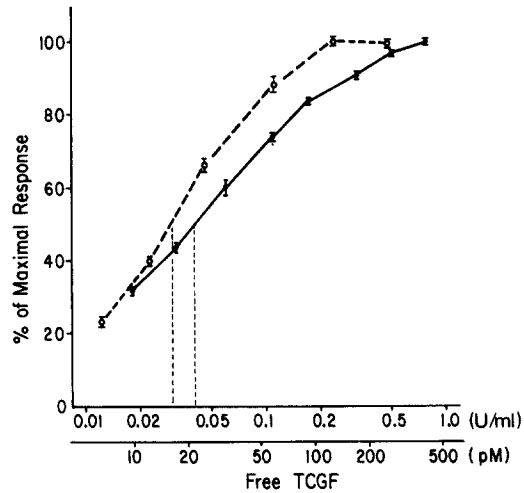

Fig. 7. Comparison between the percentages of the maximum biological response ([3H]TdR proliferation assay, CTLL-2, subclone 15H, -○-) and the maximum level of [35S]methionine-TCGF binding (CTLL-2, subclone 15H, -●-) as a function of the concentration of free TCGF. The points and brackets represent the mean and SEM of quadruplicate (-○-) or duplicate (-●-) determinations.

absorption of biological activity was found to be restricted to activated cells (10–13). The magnitude of the rise in receptor number after activation cannot be solely attributed to an increase in cell surface area as a result of blastogenesis (based on cell volume determinations, the surface area increased only 4–8-fold). Rather, the data support an actual increase in the average surface density of receptor sites. In addition, the lack of detectable [^{35}S]methionine-TCGF binding to LPS-activated B cell blasts, which were the same size as the lectin-activated blasts, not only lends support to this conclusion but also confirms that the high affinity of TCGF for activated lymphocytes is restricted to T cell blasts.

In addition to lectin- and antigen-activated cells, cloned, antigen-specific cytolytic and helper TCGF-dependent murine T cell lines also expressed significant numbers of receptor sites. In contrast to most human T cells cultured in TCGF-containing medium, which often require periodic restimulation with antigen to maintain responsiveness to TCGF (39), these murine lines have retained their sensitivity to TCGF, without restimulation, for several years in culture (3, 8). Moreover, the number of receptor sites detected on these cells has fluctuated <20% over a 6-mo period. Together with the data on antigen- and lectin-activated cells, these findings emphasize that the appearance and persistence of TCGF-specific binding sites are critical parameters for T cell clonal expansion.

The lack of receptor sites on all of the non-T cell, and most of the neoplastic T cell lines thus far examined, is consistent with their apparent lack of TCGF responsiveness. Presumably, these cell lines are either fixed in an autonomous proliferative cycle or respond to an as yet unidentified endogenous "growth" factor. The human HUT-102B2 and gibbon ape MLA-144 cell lines, however, did manifest an appreciable number of receptor sites that bound [^{35}S]methionine-TCGF with an affinity similar to that found for activated human T cell blasts. In recent studies, these two cell lines have been shown to be unique, since they appear to both produce and respond to TCGF. Although the HUT-102B2 cells release barely detectable levels of TCGF activity into the culture medium, TCGF-like activity is easily demonstrable on the cell surface as shown by acid-glycine elution (35). Furthermore, HUT-102B2 cells are responsive to TCGF, since unlike other cell lines tested, exogenous supplementation with TCGF enhances their growth rates (35). The MLA-144 cell line is somewhat different in that TCGF activity is easily detectable in the culture medium (34). That MLA-144 cells are TCGF-responsive was recently shown through studies employing glucocorticoids.[2] As has been shown for normal T cells (40, 41), glucocorticoids inhibit TCGF production by MLA-144 cells and also suppress their rate of proliferation. TCGF supplementation counteracts this effect in a concentration-dependent fashion.

The restriction of the binding of radiolabeled TCGF to T cell blasts and TCGF-dependent T cell lines, together with the finding that TCGF selects only for T cell growth (10–15), suggests that TCGF does not directly interact with B cells. Although TCGF was the prototype lymphokine for the class of biologically active molecules designated interleukin 2, subsequent observations of B cell proliferation or maturation that have been attributed to this class of molecules (42, 43) should be interpreted with caution. Such B cell responses could well be due to the effect of TCGF on residual T cells in the T-depleted cell populations used in the experiments or to components of the interleukin 2 preparations other than TCGF. In this regard, the purification of TCGF from JURKAT leukemia cells may have been aided due to the fact that these

cells, in contrast to a heterogenous normal mononuclear cell population, do not produce detectable quantities of several other biologically active moieties (e.g., CSA, B cell-stimulating factor(s), LAF) upon lectin stimulation.

The absolute values for the dissociation constant and number of binding sites per cell are dependent upon preliminary estimates of the purity and the specific activity of the radiolabeled TCGF preparations. While the JURKAT-derived [^{35}S]methionine-TCGF was reasonably uniform with respect to pI and to size on SDS-PAGE, a definitive demonstration of homogeneity requires further examination. In this regard, however, since material from each of the three distinct IEF peaks of tonsilar TCGF competed almost equally with JURKAT-derived [^{35}S]methionine-TCGF binding, it seems highly unlikely that a significant portion of the bound radiolabel could represent a copurifying contaminant. In addition, at the lowest concentrations of [^{35}S]methionine-TCGF tested on human PHA blasts, as much as 80–85% of the total radiolabel was in the bound fraction, suggesting that this is a minimum estimate of the proportion of TCGF in the preparation. The specific activity used in the calculations was based on a preliminary estimate of the relationship between biological activity and protein mass. The latter value is dependent on the purity of the unlabeled TCGF preparations and the accuracy of the biological and protein assays. Any error would result in a systematic shift in the receptor number and dissociation constant. Nevertheless, while the absolute values presented in this report may be somewhat in error, the relative abundance and apparent affinities of the receptor sites on the different types of stimulated and unstimulated cells, and the conclusions based on those relative values are unlikely to be affected.

The seeming linearity of the data when analyzed by Scatchard plots is consistent with all of the receptor sites on a given cell type having approximately the same affinity for TCGF. The dissociation constant is only an apparent one, however, since processes of internalization and/or degradation may be taking place during the time used to measure binding. If significant amounts of degraded [^{35}S]methionine TCGF were released from the cell, then the value of free TCGF assumed to correspond to equilibrium conditions would have been overestimated. Nevertheless, the data indicate, within experimental error, that receptor sites on murine Con-A and alloantigen-activated blasts and long-term T helper and cytolytic cell lines all have roughly the same apparent affinity. Similarly, the receptor sites on human PHA and alloantigen-activated blasts and HUT-102B2 cells also appear to have approximately the same affinity. The affinity of the receptor sites of the human and gibbon ape cells (MLA-144), however, were significantly higher than those of murine cells for human TCGF.

The binding of radiolabeled TCGF to murine and human cells was similar in many respects to the binding of other polypeptide hormones and factors to their target cells. Like insulin (44) and EGF (29), the binding was rapid and largely reversible. Moreover, the binding sites appeared to be on the cell surface because glutaraldehyde-fixed cells bound nearly as much [^{35}S]methionine-TCGF as viable cells and the majority of TCGF biological activity could be eluted after absorption to such fixed cells (14). As in the case of several other polypeptide factors (28–32), radiolabeled TCGF underwent degradation that was time, temperature, and receptor dependent. In addition, as with insulin (32), EGF (28, 29), and human growth hormone (30, 31), degradation was blocked by the lysosomotropic agents, ammonium chloride and chloroquine. By analogy to these and other polypeptide factors (45), the cellular

degradation of TCGF can be expected to involve internalization by adsorptive pinocytosis and lysosomal association.

Besides serving as a means of detecting cellular receptors, the binding assay can be used as in Fig. 6 as a competitive assay for unlabeled TCGF. Compared with the biological assay of proliferation, the competitive assay is faster (1 vs. 24 h for murine CTLL or 48 h for human target cells) and should avoid some of the complications of nonspecific inhibitors of cell division and metabolism that affect the proliferation assay. Moreover, increasing the specific activity of the radiolabeled TCGF by increasing the ratio of labeled to unlabeled amino acids in the original culture or by improving the efficiency of their incorporation, should enhance the sensitivity of the competitive assay (about 0.3 U TCGF/ml) by allowing the use of a lower molar concentration of radiolabeled TCGF. In this regard, attempts to prepare high specific activity, radioiodinated TCGF have thus far met with disappointing results (Dr. Richard Robb, unpublished observations). A significant portion of the TCGF biological activity was consistently lost during the labeling process, a fact which would complicate evaluation of the subsequent binding data. Internally radiolabeled TCGF has an advantage in that the radiolabeled molecules behave identically with unlabeled molecules in associating with the celluar receptor (as determined by substituting various amounts of unlabeled TCGF for [^{35}S]methionine-TCGF during the binding assay). In addition, the ^{35}S-, and especially the ^{3}H-, substituted molecules have a much longer half-life than iodinated preparations.

The biological relevance of the TCGF receptor sites described in this report is emphasized by the close correlation between the concentrations of TCGF giving rise to a proliferative response (16 pM free TCGF mediated half-maximal proliferation of TCGF-dependent CTLL cells) and the concentrations leading to significant occupation of receptor sites (21 pM free [^{35}S]methionine-TCGF led to half-maximal binding). This finding provides an explanation for the strict TCGF concentration-dependence of T cell proliferation that serves as the quantitative basis for the biological TCGF assay. Moreover, because TCGF appears to be degraded once TCGF-receptor binding occurs, it necessarily follows that the dynamics of TCGF-receptor turnover in part determines the concentration of TCGF available to proliferating T cells. As a result of these findings, it may be anticipated that the extent of in vitro, and most probably in vivo, T cell clonal expansion after lectin or antigen activation is determined by the TCGF concentration, the acquisition and persistence of TCGF-specific receptors, the affinity of the receptors for TCGF, and the rate of degradation of TCGF and its receptor. It appears, therefore, that an endocrinological approach to the study of TCGF and its receptor will provide not only new insight important for the in vitro cultivation of T cells, but possibly new diagnostic and therapeutic approaches for dealing with disorders that involve T cells.

Summary

To examine directly the hypothesis that T cell growth factor (TCGF) interacts with target cells in a fashion similar to polypeptide hormones, the binding of radiolabeled TCGF to various cell populations was investigated. The results indicate that TCGF interacts with activated T cells via a receptor through which it initiates the T cell proliferative response.

Internally radiolabeled TCGF, prepared from a human T leukemia cell line and

purified by gel filtration and isoelectric focusing, retained biological activity and was uniform with respect to size and charge. Binding of radiolabeled TCGF to TCGF-dependent cytolytic T cells occurred rapidly (within 15 min at 37°C) and was both saturable and largely reversible. In addition, at 37°C, a receptor- and lysosome-dependent degradation of TCGF occurred. Radiolabeled TCGF binding was specific for activated, TCGF-responsive T cells. Whereas unstimulated lymphocytes of human or murine origin and lipopolysaccharide-activated B cell blasts expressed few if any detectable binding sites, lectin- or alloantigen-activated cells had easily detectable binding sites. Moreover, compared with lectin- or alloantigen-activated T cells, long-term TCGF-dependent cytolytic and helper T cell lines and TCGF-dependent neoplastic T cell lines bound TCGF with a similar affinity (dissociation constant of 5–25 pM) and expressed a similar number of receptor sites per cell (5,000–15,000). In contrast, a number of TCGF-independent cell lines of T cell, B cell, or myeloid origin did not bind detectable quantities of radiolabeled TCGF. Binding of radiolabeled TCGF to TCGF-responsive cells was specific, in that among several growth factors and polypeptide hormones tested, only TCGF competed for binding. Finally, the relative magnitude of T cell proliferation induced by a given concentration of TCGF closely paralleled the fraction of occupied receptor sites. As the extent of T cell clonal expansion depends on TCGF and on the TCGF receptor, the dissection of the molecular events surrounding the interaction of TCGF and its receptor that these studies permit, should provide new insight into the hormonelike regulation of the immune response by this lymphokine.

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