Cellular receptor for ^{125}I -labeled tumor necrosis factor: Specific binding, affinity labeling, and relationship to sensitivity

(immunomodulator/inflammation/macrophage cytotoxin/necrosin)

FREDERICK C. KULL, JR., STEVEN JACOBS, AND PEDRO CUATRECASAS

Molecular Biology Department, The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709

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ABSTRACT Tumor necrosis factor (TNF) is a proteinaceous toxin shed by stimulated myeloid cells. Murine TNF was radioiodinated to a specific activity of 1 mCi/nmol (1 Ci = 37 GBq) of monomer. ^{125}I -labeled TNF (^{125}I -TNF) retained complete cytotoxic activity and it was immunochemically identical to the native toxin in a quantitative immunoprecipitation assay. It could be shown by competition binding that ^{125}I -TNF bound to intact L929 cells with a specificity equal to that of native toxin. The conditions of time, temperature, and concentration involved in equilibrium specific binding to intact cells were studied in detail. When binding was carried out at 4°C for 18 hr, four cell lines sensitive to ^{125}I -TNF cytotoxicity demonstrated high-affinity binding. The binding reached half-maximal level at 3 pM and saturated at 30 pM. These concentrations approximated those required for cell death. Scatchard analysis gave ≈ 1000 sites per cell. J774.1 cells, the source of the toxin, demonstrated similar binding but were not sensitive to ^{125}I -TNF cytotoxicity. Other sensitive cell lines and freshly extracted tumor cells showed specific binding at 3 pM. Normal lymphoid organ cell suspensions and two human tumorigenic cell lines were not sensitive and failed to demonstrate specific binding. ^{125}I -TNF, covalently cross-linked to its receptor on sensitive L-M cells with disuccinimidyl suberate, was isolated and analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and autoradiography. Two specific bands were identified. The most prominent band had a mobility corresponding to a molecular mass of 95 kDa and the second band had a molecular mass of 75 kDa. The presence of the binding site appears to be necessary but not sufficient to explain the sensitivity of cells to the cytotoxic action of TNF.

Myeloid cells shed a potent toxin when they contact small concentrations of bacterial lipopolysaccharide. The toxin is called macrophage cytotoxin or tumor necrosis factor (TNF). It has been purified to homogeneity from the supernatants of stimulated murine (1) and human (2, 3) macrophage-like cell lines. The purified protein is cytotoxic to sensitive cell lines at picomolar concentrations (1–3), and picomolar amounts of the natural murine (unpublished work), human, or human recombinant gene product (3–5) have the capacity to induce the necrosis of solid tumor transplants in mice.

The toxin is one of two structurally related products shed by stimulated leukocytes since, considering conservative amino acid substitutions, it shares $\approx 50\%$ amino acid sequence homology with lymphotoxin (2), a lymphoblastoid cell product that possesses similar biologic properties (6). The biologic activities of these toxins were originally attributed to an endogenous necrotizing factor called necrosin (7). The role that these substances play in infection, inflammation, or immunity is not currently appreciated but it seems reasonable to postulate that they are immunomodulators.

No direct causative mechanisms have been identified that explain the toxins' extraordinary properties. *In vitro* evidence with the myeloid toxin suggests that target cells contribute to their own demise. Active cellular metabolism and lysosomal function were required for cell death and we have speculated that internalization of the toxin may be required for cell death (8). Here we describe a high-affinity binding site for the murine toxin TNF on the surface of diverse target cells. The presence of the receptor appears to be necessary but insufficient to explain the sensitivity of the target cells to the cytotoxic activity of murine TNF.

MATERIALS AND METHODS

Cell Lines and Culture. The mouse tumorigenic fibroblast lines L-M (CCL 1.2) and L929 (CCL 1), the murine nontumorigenic fibroblast BALB/3T3 (clone A31) (CCL 163), CPAE (CCL 209), a bovine aortic endothelial line, and IM-9 (CCL 159), a human B-lymphoblast line, were obtained from the American Type Culture Collection. The following lines were gifts from the indicated individuals: BCE, bovine capillary endothelial (J. Folkman); PAE, pig aortic endothelial (H. Berger); HL60, human promyelocytic leukemia (R. Gallo); HepG2, human liver carcinoma (B. Knowles); MCF7, human mammary carcinoma (I. Parikh); H35, rat liver carcinoma (I. Goldfein); J774.1, murine macrophage-like (Y. Gillespie). The lines were cultured in standard medium formulations with 5–10% supplemented calf serum (Sterile Systems, Logan, UT) as described (1). The murine transplantable tumors Meth A and P388 were obtained from the Trudeau Institute. They were maintained as ascites tumors in CD2F1 mice (Charles River Breeding Laboratories). B6D2 mice were obtained from the Trudeau Institute.

TNF. Murine macrophage cytotoxin (TNF) was extracted from the supernatants of J774.1 cells and purified as described (1). Two preparations were used. The first was purified to electrophoretic homogeneity and had a specific activity of 3×10^4 cytotoxic units/ μg of protein. (Cytotoxic units were assessed on L-M cells as detailed in the legend to Fig. 2). It was radiolabeled as described below. The second preparation, which served to determine nonspecific binding, had a specific activity of 100 units/ μg .

TNF was radioiodinated by mixing 2 μg of the purified protein with 1 mCi (1 Ci = 37 GBq) of carrier-free ^{125}I (CintiChem) in 100 μl of 25 mM Tris/180 mM glycine, pH 8.0, in an Iodo-Gen- (Pierce) coated glass tube for 20 min at 0°C. The reaction was quenched with 100 nmol of sodium metabisulfite followed by 100 nmol of tyrosine. It was diluted with 300 μl of 0.1% bovine serum albumin and the mixture was percolated through a Sephadex G-25 (Pharmacia) column equilibrated and run with 20 mM NaP₆/140 mM NaCl/0.1% bovine serum albumin. ^{125}I -labeled TNF (^{125}I -TNF) eluted in the void volume peak of radioactivity. It was purified free of

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Abbreviations: TNF, tumor necrosis factor; ^{125}I -TNF, ^{125}I -labeled TNF.

other radiolabeled components by HPLC ion exchange. HPLC ion exchange, HPLC gel filtration, and sodium dodecyl sulfate/polyacrylamide gel electrophoresis (Laemmli system, ref. 9) were carried out as described for unlabeled native toxin (1). Protein molecular weight standards were obtained from Bethesda Research Laboratories and Sigma. Radioactivity was determined in a Packard gamma scintillation spectrometer whose efficiency (75%) was monitored regularly. Autoradiography was carried out with X-Omat AR film (Kodak) with a Cronex Lightning Plus intensifying screen (DuPont). Immunoprecipitation using rat anti-mouse TNF antiserum was carried out by an unpublished method.

Affinity Cross-Linking. Disuccinimidyl suberate (Pierce) was freshly prepared as a 20 mM solution in dimethyl sulfoxide. ^{125}I -TNF was cross-linked to itself by diluting disuccinimidyl suberate to 0.2 mM in 1 ml of 140 mM NaCl containing 40 fmol ^{125}I -TNF. The reaction was left to stand at 0°C for 45 min and quenched with 1 mmol of sodium acetate. ^{125}I -TNF was cross-linked to intact L-M cells essentially as described for murine colony-stimulating activity (10). Briefly, L-M monolayers were incubated overnight at 4°C with 10 pM ^{125}I -TNF. The cells were washed free of unbound label and left to sit for 45 min at 0°C in 0.2 mM disuccinimidyl suberate/140 mM NaCl. The reaction was quenched with sodium acetate and the cells were washed as described (10). The cells were solubilized in 1% NaDodSO₄ and analyzed by NaDodSO₄/PAGE.

RESULTS

Ligand Efficacy. TNF was iodinated to a specific activity of 1 mCi/nmol of monomer, which was equivalent to an average of one ^{125}I atom per dimer. It migrated on HPLC ion-exchange and HPLC gel filtration similarly to unlabeled toxin (data not shown). Upon autoradiography of NaDodSO₄/polyacrylamide gels, it appeared as a single radioactive band that migrated similarly to the purified native toxin (Fig. 1). ^{125}I -TNF was fully active biologically as assessed by cytotoxicity on L-M cells (Fig. 2). It was immunochemically identical to the native toxin in an immunoprecipitation assay using rat anti-TNF antiserum (data not shown). The native protein is composed of non-sulfhydryl-linked dimers, trimers, and tetramers of a ≈ 17 -kilodalton (kDa) monomer (1–5). The apparent size of the labeled toxin was 40 kDa as assessed by gel filtration under nondenaturing conditions. Chemical cross-linking studies (see below) suggested that the prominent form may be a dimer. Hence, the value 34 kDa was used to calculate molarity (specific activity = 4650 dpm/fmol). The capacity of native TNF to compete with ^{125}I -TNF for binding to L929 cells was compared (Fig. 3). When binding was carried out with 5 pM ^{125}I -TNF (a near saturating concentration) and increasing concentrations of native TNF, half the labeled binding was inhibited by 5 pM native TNF; thus, the toxins appeared to share the same affinity.

Equilibrium Specific Binding. ^{125}I -TNF bound to sensitive target cells in a specific manner; that is, the labeled ligand was displaced by a 200-fold excess of unlabeled toxin. Temperature and time course experiments (data not shown) indicated that the specific binding achieved equilibrium in 4–6 hr at 23°C and in 12–20 hr at 4°C. Equilibrium was not achieved at 37°C. The specific binding peaked in 30 min and declined sharply thereafter; hence, binding experiments were carried out at 4°C. The equilibrium specific binding of ^{125}I -TNF to L-M cells is shown in Fig. 4. The binding was saturable at 30 pM and was half-maximal at 3 pM. Other noteworthy features of the binding included low nonspecific binding and good precision; for example, virtually all of the radioactivity bound at 10 pM ^{125}I -TNF (1660 dpm per 3×10^5 cells) was specific. It was displaced by 3 nM unlabeled TNF. The standard error of the mean for triplicate measurements was ± 70 dpm.

FIG. 1. NaDodSO₄/PAGE. Purified native TNF (left lane) and ^{125}I -TNF (right lane) were electrophoresed under identical conditions in 15% acrylamide gels (Laemmli system). The left lane, stained by the silver stain method (11), contained 100 ng of protein. The autoradiogram, right lane, was obtained from 20 pg of protein (2700 dpm). Molecular mass standards were run simultaneously: ovalbumin, 43 kDa; α -chymotrypsinogen, 25.7 kDa; β -lactoglobulin, 18.4 kDa; lysozyme, 14.3 kDa; bovine trypsin inhibitor, 6.2 kDa; insulin chains, 3 kDa.

Scatchard analysis (ref. 13; see Fig. 4 *Inset*) gave a value of 1100 binding sites per cell. The equilibrium specific binding on other sensitive cell lines was examined in a similar fashion. In each of these the Scatchard plots were linear and the K_d was 3 pM, but they differed modestly (less than 2-fold) in their number of binding sites. The results are presented in Table 1.

Correlation with Sensitivity. Table 1 shows that the K_d for binding is in the range of concentration required to kill half the cells during an 18-hr incubation. J774.1, which was the source of the toxin, was an exception. It was not sensitive to the cytotoxic capacity of the toxin. Single-point equilibrium

FIG. 2. Cytotoxicity of ^{125}I -TNF. Serial log₃ dilutions of test material in Eagle's minimal essential medium were mixed in duplicate in microtiter wells containing 3.2×10^4 growing L-M cells. The cultures were incubated at 38°C for 18 hr and scored for number of viable cells by neutral red uptake (OD₅₄₂) as detailed elsewhere (12). Points are means for duplicate wells. $\circ$, ^{125}I -TNF (0 dilution = 1 ng per well); $\bullet$, common standard (0 dilution = 33 units per well). The specific activity of the prelabeled preparation, as measured against the common standard, was 30 units/ng.

FIG. 3. Binding competition of L929 cells. Cells were seeded at 10^6 per ml of growth medium per 9-cm² well and incubated overnight at 37°C. They were washed three times with cold binding medium (Eagle's minimal essential medium/1% calf serum/10 mM HEPES) and refrigerated at 4°C for 18 hr with 1.6 ml of binding medium per well containing 5 pM (8 fmol = 25,000 cpm) ¹²⁵I-TNF with and without the indicated concentration of unlabeled TNF. Cells were washed three times with cold binding medium and solubilized with 0.5 ml 1% NaDodSO₄, and radioactivity was measured. Points are means for duplicate wells. The maximum deviation of any measurement was <5% of the average value.

binding was determined for a number of other cultured cell lines. The lines differed with respect to species, tissue of origin, and sensitivity to the cytotoxic activity of TNF. Also included were two murine transplantable tumors and two murine normal lymphoid organ cell suspensions. The sensi-

FIG. 4. Equilibrium saturation binding to L-M cells. Cells were seeded at 1.5×10^4 per ml of growth medium per 2-cm² well, incubated, and prepared for binding as described in the legend to Fig. 2. They were then refrigerated at 4°C for 18 hr with the indicated concentration of ¹²⁵I-TNF with (nonspecific) and without (total) 3 nM unlabeled TNF, washed, and treated as described in the legend to Fig. 2. Results represent specific binding (mean total - mean nonspecific) $\pm$ SEM for total binding. Each experiment was carried out in triplicate. B, bound; F, free.

Table 1. K_d , number of binding sites per cell, and lethal concentration

Cell line (origin)	K_d , pM	Sites per cell	LC ₅₀ , pM
L-M (murine tumorigenic fibroblast)	3	1100	2
L929 (murine tumorigenic fibroblast)	3	770	4*
3T3A31 (murine nontumorigenic fibroblast)	2.5	1050	2.5
J774.1 (murine macrophage-like)	3	1100	>200*
CPAE (bovine aortic endothelium)	3	1500	30

Equilibrium saturation binding curves were determined on the indicated cell lines as described for L-M cells in the legend to Fig. 4. CPAE were seeded at 10^5 /ml per well. Scatchard plots were then composed as shown in Fig. 4. The K_d was the concentration that gave half-maximal binding, and the number of binding sites per cell was obtained from the x intercept. LC₅₀, concentration of toxin required to kill half the cells using the conditions of our standard survival assay (see legend to Fig. 2).

*Measured in the presence of 4 μ M cycloheximide.

tivity of L-M, CPAE, and MCF₇ and the insensitivity of HepG2 have been shown previously (1). Sensitivity (discussed in ref. 8) was assessed in a somewhat arbitrary fashion, because the variety of cells necessitated a range of assay conditions (media, serum concentration, adherence, concentration of toxin, length of exposure, or presence of cycloheximide). Sensitivity is hereby defined as at least 50% cell death (compared with untreated controls) in the presence of less than 200 pM TNF in the routine growth medium of the cells for some time up to 72 hr of culture $\pm$ a sublethal concentration of cycloheximide (0.1–1 μ g/ml). As shown in Table 2, the majority of cells specifically bound ¹²⁵I-TNF. These included reputed tumorigenic and nontumorigenic lines and two transplantable tumors. All the cells that demonstrated binding were sensitive. Two human lines (IM-9, a B lymphoblastoid, and HepG2, a liver carcinoma) and freshly extracted mouse splenocytes and thymocytes failed to show appreciable specific binding. These cells were not sensitive to the cytotoxic effects of the toxin.

Affinity Cross-Linking. ¹²⁵I-TNF was cross-linked to intact

Table 2. Single-point equilibrium specific binding

Line (origin)	cpm/ 10^6 cells	Sensitive
L-M (murine)*	1625 $\pm$ 58	+
3T3A31 (murine)*	3686 $\pm$ 117	+
P388 (murine)	2298 $\pm$ 590	+
Meth A (murine)	1878 $\pm$ 232	+
Splenocytes (murine)	129 $\pm$ 62	—
Thymocytes (murine)	54 $\pm$ 20	—
H35 (rat)*	1530 $\pm$ 86	NT
BCE (bovine)*	715 $\pm$ 130	+
PAE (porcine)	944 $\pm$ 68	+
HL60 (human)	1625 $\pm$ 860	+
MCF ₇ (human)*	744 $\pm$ 58	+
HepG2 (human)*	10 $\pm$ 1	—
IM-9 (human)	0 $\pm$ 37	—

Binding to adherent cell lines (* = adherent) was carried out as described for L-M cells in the legend to Fig. 2, except that the cells were seeded at 5×10^5 per 9-cm² wells. Washed suspension cell lines were maintained at 10^6 cells per 1.6 ml. All were refrigerated at 4°C for 18 hr with 3 pM (15,000 cpm/1.6 ml) ¹²⁵I-TNF in the absence (total) or presence (nonspecific) of 3 nM unlabeled TNF. Cells were washed three times in cold binding medium and specific binding was determined as described in the legend to Fig. 2. Results are normalized to 10^6 cells. The SEM for adherent lines was <10% of the mean value, and for suspended cells, it was 20%. Sensitivity was evaluated as described in the text. Each experiment was carried out in triplicate. NT, not tested.

L-M cells with disuccinimidyl suberate (which covalently coupled the adjacent amino groups). The cell solute was analyzed by autoradiography following NaDodSO₄/PAGE as shown in Fig. 5. Specific bands were observed at 95 kDa, 30 kDa, and the dye front. In addition, a very faint band was observed at 75 kDa. The 75-kDa band is barely discernible from the photograph of Fig. 5. No nonspecific bands were apparent. Most of the radioactivity was associated with the dye front and 5% of the radioactivity was associated with the 30-kDa band. These bands were likely due to toxin that did not couple to intact cells (discussed below). The 95-kDa band was the most prominent one that resulted from the proximity of the ligand to intact cells. Five percent of the radioactivity was associated with this band. In a second trial (not shown), 13% of the total radioactivity was associated with the 95-kDa band. This and subsequent trials showed the persistent presence of the faint 75-kDa band.

As shown in Fig. 5, ¹²⁵I-TNF migrated with the dye front in a 7.5% polyacrylamide gel. Cross-linked to itself, it demonstrated a major band at the dye front, a band at 30 kDa, and a faint band at 45 kDa. These bands were consistent with monomers (dye front), dimers (30 kDa), and trimers (45 kDa). Their relative intensity reflects both the stoichiometry of the intact toxin (which migrated in gel filtration as a 40-kDa mass and is probably primarily a dimer) and the inefficiency of coupling. No higher molecular mass bands were apparent. The bands exhibited slightly lower mobilities (higher molecular mass) when electrophoresed under reducing conditions. The lower mobilities suggested the presence of intramolecular disulfide bonds. (The sequence of human TNF reveals two cysteines that may pair, refs. 3–5.)

FIG. 5. Chemical cross-linking depicted by autoradiography of NaDodSO₄/polyacrylamide gels. ¹²⁵I-TNF alone and ¹²⁵I-TNF bound to intact L-M cells were treated with disuccinimidyl suberate as described in the text. Samples were applied to a 7.5% acrylamide gel (Laemmli system). Lanes: 1, ¹²⁵I-TNF; 2 and 3, ¹²⁵I-TNF cross-linked to itself; 4 and 5, ¹²⁵I-TNF cross-linked to intact L-M cells in the absence or presence of 3 nM unlabeled TNF. + and -, presence or absence of 2-mercaptoethanol in the loading buffer. The molecular mass scale was derived from the relative mobilities of the following standards, which were run simultaneously: myosin, 205 kDa; β -galactosidase, 116 kDa; phosphorylase B, 97 kDa; albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 29 kDa.

DISCUSSION

We have identified a high-affinity binding site for ¹²⁵I-TNF on the surface of cells that were sensitive to its cytotoxic activity. Two criteria suggest that this binding site is the physiologic receptor for TNF. First, the ligand used in our studies mimicked the native toxin in all ways examined: it retained full cytotoxic activity, it was equally competitive with native toxin for binding to cultured cells, it retained immunochemical cross-reactivity, and it migrated like the native toxin in gross separation techniques (gel filtration and ion-exchange chromatography and NaDodSO₄/PAGE).

Second, the concentrations that were toxic to target cells were approximately those that saturated or half-saturated their receptors. Indeed, the conditions for binding (cell density and concentration of ligand) were chosen because of our experience with the cytotoxic capacity of the toxin; for example, L-M and L929 are the most common target cell lines used by investigators for the measurement and study of cytotoxicity (8, 14–16). The concentration of toxin that killed half of these cells in an overnight culture was approximately the K_d for binding (3 pM). 3T3A31, a nontumorigenic fibroblast, exhibited binding and sensitivity similar to that of L-M. CPAE, a diploid bovine aortic endothelial line, required a saturating dose to kill half the cells (30 pM). The pM concentration range may also be physiologic with respect to production of the toxin. J774.1, other murine macrophage-like lines (17), and adherent peripheral blood leukocytes (unpublished observation similar to ref. 18) at 10⁶ cells per ml shed pM concentrations of the toxin when they are stimulated with lipopolysaccharide. Binding to J774.1 was measured when the cells were unstimulated and production of the toxin could not be detected.

The specific binding curves were determined at 4°C when binding had reached equilibrium. Although the binding was saturated at 30 pM and half-maximal at 3 pM, theoretical considerations show that the actual K_d was probably lower (the concentration of binding sites was close to the K_d , see ref. 19). The Scatchard plots were consistent with a single class of high-affinity binding sites with approximately 1000 sites per cell. In comparison with receptors for other lymphokines, the affinity was somewhat high and the number of binding sites was somewhat low; for example, the K_d and number of sites per cell for interleukin 2 receptors have been estimated at 20 pM and 9000, respectively (20). The quantities of material available to us precluded testing for low-affinity TNF binding sites.

An assortment of other cells bound TNF at low concentration (3 pM) and 4°C. These cells were also sensitive to the cytotoxic activity of TNF, and some have been used in the study of its properties. The sensitivity of some, like L929, required the concomitant presence of cycloheximide. DNA, RNA, and protein synthesis inhibitors are known to enhance sensitivity (8) and they are commonly incorporated into routine assays for lymphotoxin (6) and TNF (2, 21). The HL60 line was a source of human TNF (2–4). The Meth A transplantable tumor has been used to demonstrate the *in vivo* tumor-necrotizing activity of TNF (ref. 4 and unpublished work). Two human tumorigenic lines (HepG2 and IM-9) showed negligible specific binding and were not sensitive to TNF cytotoxicity even in the presence of cycloheximide. Since we use these lines for the study of insulin-like growth factor 1 receptors, these results suggest that the insulin-like growth factors would not compete with TNF. Thymocytes and splenocytes showed a small amount of binding and a subpopulation of lymphocytes could be responsible for most of the binding (22, 23). Our results with cultured macrophage-like lines suggest that monocytes might show specific binding.

While high-affinity specific binding appears to correlate with sensitivity, binding alone is not sufficient to explain cytotoxicity. Cell lines may differ in their capacity to repair the fatal lesions. Alternatively, they may differ in the concentration of an intracellular substrate for the toxin. Internalization of the toxin may be required for cell death, since numerous treatments known to affect aspects of receptor-mediated internalization and degradation decrease the sensitivity of target cells (6).

Disuccinimidyl suberate, which covalently couples adjacent amino groups, was employed for the molecular characterization of the binding site. This reagent has been used extensively to identify the cellular receptors for a number of hormones such as insulin (24) and glucagon (25). Our studies presented some technical difficulty because the number of binding sites was so small and because cross-linking was inefficient. The electrophoresis gels were heavily loaded with cell extract, and the autoradiography exposure times were long. Two specific cell-associated bands were observed. The results are subject to a number of interpretations. Since the 95-kDa band was the most prominent band, we speculate that this represents the receptor complex. We could not distinguish whether monomeric or dimeric ^{125}I -TNF was cross-linked to either cellular band. Because cross-linking was inefficient, we speculate that the 95-kDa band represents the cross-linking of labeled monomer to an ≈ 80 -kDa polypeptide. However, the dimer may be cross-linked to an ≈ 65 -kDa polypeptide. The polypeptide may be a noncovalently bound subunit of a more complex molecule. The faint 75-kDa band could be a part of the molecular milieu of such a complex. It may represent a distinct subunit whose amino groups are less favorably positioned for cross-linking. Alternatively, it may be a precursor or degradation product of the 95-kDa band or it may be a unique receptor. Both bands exhibited slightly higher molecular masses when electrophoresed under reducing conditions (data not shown). Their decreased mobility could be accounted for by the change in the ligand. Neither band contained sulfhydryl-linked subunits.

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1. Kull, F. C., Jr., & Cuatrecasas, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7932-7936.
2. Aggarwal, B. B., Kohr, W. J., Hass, P. E., Moffat, B., Spencer, S. A., Henzel, W. J., Bringman, T. S., Nedwin, G. E., Goeddel, D. V. & Harkins, R. N. (1985) *J. Biol. Chem.* **260**, 2345-2354.
3. Wang, A. M., Creasey, A. A., Ladner, M. B., Lin, L. S., Strickler, J., Van Arsdel, J. N., Yamamoto, R. & Mark, D. F. (1985) *Science* **228**, 149-154.
4. Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynk, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B. & Goeddel, D. (1984) *Nature (London)* **312**, 724-729.
5. Shirai, T., Yamaguchi, H., Ito, H., Todd, C. W. & Wallace, R. B. (1985) *Nature (London)* **313**, 803-806.
6. Gray, P. W., Aggarwal, B. B., Benton, C. V., Bringman, T. S., Henzel, W. J., Jarett, J. A., Leunig, D. W., Moffat, B., Ng, P., Svedersky, L. P., Palladino, M. A. & Nedwin, G. E. (1984) *Nature (London)* **312**, 721-724.
7. Menkin, V. (1956) *Biochemical Mechanisms in Inflammation* (Charles C Thomas, Springfield, IL), pp. 195-231.
8. Kull, F. C., Jr., & Cuatrecasas, P. (1981) *Cancer Res.* **41**, 4885-4890.
9. Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685.
10. Morgan, C. J. & Stanley, E. R. (1984) *Biochem. Biophys. Res. Commun.* **119**, 35-41.
11. Merrill, C. R., Goldman, D., Sedman, S. A. & Ebert, M. H. (1981) *Science* **211**, 1437-1438.
12. Kull, F. C., Jr., & Cuatrecasas, P. (1983) *Appl. Biochem. Biotech.* **8**, 97-103.
13. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660-672.
14. Matthews, N. & Watkins, J. F. (1978) *Br. J. Cancer* **38**, 302-309.
15. Ruff, M. R. & Gifford, G. E. (1981) *Infect. Immun.* **31**, 380-385.
16. Williamson, B. D., Carswell, E. A., Rubin, B. Y., Prendergast, J. S. & Old, L. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5397-5401.
17. Kull, F. C., Jr., & Cuatrecasas, P. (1983) in *Interleukins, Lymphokines, and Cytokines*, eds. Cohen, S. & Oppenheim, J. (Academic, New York), pp. 511-520.
18. Matthews, N. (1978) *Br. J. Cancer* **38**, 310-315.
19. Cuatrecasas, P. & Hollenberg, M. D. (1976) *Adv. Protein Chem.* **30**, 251-451.
20. Robb, R. J., Munck, A. & Smith, K. A. (1981) *J. Exp. Med.* **154**, 1455-1474.
21. Flick, D. A. & Gifford, G. E. (1984) *J. Immunol. Methods* **68**, 167-175.
22. Umeda, T., Hara, T. & Nijima, T. (1983) *Mol. Cell. Biol.* **29**, 349-352.
23. Playfair, J. H. L., de Souza, J. B. & Taverne, J. (1982) *Clin. Exp. Immunol.* **47**, 753-755.
24. Pilch, P. F. & Czech, M. P. (1979) *J. Biol. Chem.* **254**, 3375-3381.
25. Johnson, G. L., MacAndrew, V. I., Jr., & Pilch, P. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 875-878.