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Immunology

Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies

(HL-60 cells/U-937 cells/tumor necrosis factor binding protein/cachectin/lymphotoxin)

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ABSTRACT **The pleiotropic cyto/lymphokine tumor necrosis factor (TNF) exerts its functions by binding to specific cell-surface receptors. We have prepared two sets of monoclonal antibodies (mAbs) against TNF-binding proteins from the HL-60 (htr-mAb series) and U-937 (utr-mAb series) cell lines. The htr antibodies inhibit the binding of ^{125}I-labeled TNF-α to HL-60 cells only partially, whereas they block the TNF-α binding to several adenocarcinoma cell lines (HEp-2, HeLa, and MCF7) almost completely. In contrast, the utr antibodies have no effect on TNF-α binding to the adenocarcinoma cell lines but partially inhibit TNF-α binding to HL-60 and U-937 cells. However, htr-9 and utr-1 antibodies in combination fully inhibit the TNF-α binding to HL-60 and U-937 cells. The binding of TNF-β to HEp-2 and U-937 cells is also inhibited by htr and utr antibodies. Neither htr nor utr mAb has an effect on the TNF-sensitive murine cell lines L929 and WEHI 164. Flow cytometry studies show that mAbs htr-9 and utr-1 detect two distinct TNF-binding sites on human cell lines. Immunologic blot and immunoprecipitation analyses indicate that mAbs htr-9 and utr-1 recognize proteins of ≈55 kDa and 75 kDa, respectively. These data provide evidence for the existence of two distinct TNF receptor molecules that contribute to varying extent to the TNF binding by different human cells.**

Tumor necrosis factor (TNF) was originally discovered as a serum protein with necrotizing effect on certain transplantable mouse tumors *in vivo* (1) and cytotoxic effects against some transformed cells *in vitro* (2). It is mainly produced by activated macrophages and lymphocytes. Because recombinant TNF-α in later studies was found to have many additional activities *in vivo* and *in vitro*, TNF is now considered a pleiotropic cyto/lymphokine with regulatory functions in immune and inflammatory reactions (3, 4). It was found to have beneficial effects in mycobacterial infection (5) and in spontaneous autoimmune glomerulonephritis in mice (6). Based on results from animal experiments TNF is believed to play a key role in a number of pathological conditions such as septicemia (7), cachexia (8), and cerebral malaria (9).

TNF-α has been shown by x-ray diffraction studies to be a tightly packed trimer (10). TNF-β is 28% homologous with TNF-α (11) and competes with TNF-α for cellular TNF-binding sites (12). Several investigators have identified TNF receptors at the surface of a variety of cell types. A single class of high-affinity sites with K_d in the range of 0.1–1.0 nM and a few hundred to a few thousand copies per cell has been reported (13–15). The approximate molecular size of the TNF-α-binding proteins, as determined in crosslinking studies, varies from ≈50 kDa to 140 kDa (15, 16). More recently, biochemical evidence for the existence of two major TNF-α-binding proteins has been reported (17). Due to the lack of specific reagents it was not possible to analyze the TNF-binding proteins in more detail or to assign a function to the individual receptor molecules. Here we describe the preparation of two sets of noncrossreacting antibodies that recognize two separate TNF-binding proteins expressed to various relative amounts on different cell lines. Both antigens bind TNF-α and TNF-β specifically with high affinity.

MATERIALS AND METHODS

Cells and Reagents. A subclone of U-937 cells, designated U-937/T, which overexpresses TNF receptor was provided by T. Espevik (Trondheim, Norway). All other cell lines were originally from the American Type Culture Collection and were cultured continuously in RPMI 1640 (GIBCO) with glutamine, bicarbonate, penicillin, streptomycin, and 10% horse or fetal bovine serum at 5% carbon dioxide. Purified TNF-α (17) and TNF-β from *Escherichia coli* (cytotoxic activity $>10^7$ units/mg) were 99% pure as judged by SDS/PAGE. ^{125}I-labeling with Iodo-Gen (Pierce; ref. 18) resulted in an incorporation of 10–25 GBq/μmol for TNF-α and 8 GBq/μmol for TNF-β. Biotinylation of antibodies was performed according to ref. 19.

Antigen, Immunization, Fusion, and Screening. TNF-binding proteins were partially purified by ligand affinity chromatography. Briefly, the lysate from 3×10^{10} HL-60 cells or from 5×10^{10} U-937/T cells was loaded on a TNF-α–Sepharose 4B affinity column. Proteins eluted at pH 2.5 were further fractionated by reversed-phase HPLC. Aliquots of the fractions eluted were tested for ^{125}I-TNF-α-binding activity in dot blot assays on nitrocellulose membranes (Bio-Rad), and active fractions were pooled and concentrated. Each antigen (10 μl) was emulsified with complete Freund's adjuvant (15 μl) and injected into one rear foot of an anesthetized BALB/c mouse in 5-μl aliquots at weekly intervals. The popliteal lymph node was taken 2 days after the last injection, and the cells were fused to PAI myeloma cells (20), according to standard procedures. Antibodies were selected from 500 hybridomas [htr monoclonal antibodies (mAbs)] and 800 hybridomas (utr mAbs) by coprecipitation of ^{125}I-TNF-α with the receptor, by inhibition of ^{125}I-TNF-α binding to cell lines, by dot blot assays, and by immunologic blotting with purified antigen. Hybridomas were subcloned, and antibodies were purified from spent culture medium by affinity chromatography on Sepharose 4B conjugated with affinity-purified anti-mouse immunoglobulin from rabbit (RamIg). Isotypes were determined by immuno-

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Abbreviations: TNF, tumor necrosis factor; TNF-α, recombinant human tumor necrosis factor α, cachectin; TNF-β, recombinant human lymphotoxin; ^{125}I-TNF-α, ^{125}I-labeled TNF-α; ^{125}I-TNF-β, ^{125}I-labeled TNF-β; BSA, bovine serum albumin; mAb, monoclonal antibody; RamIg, affinity-purified anti-mouse immunoglobulin from rabbit.

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diffusion against type-specific sera (Nordic, Tilburg, The Netherlands). None of the mAbs immunoprecipitated ^{125}I-TNF-α.

TNF-Binding Assay. Adherent cells ($\approx 5 \times 10^5$) were assayed in 15-cm^2 dishes at 90% confluency, and suspension cells (5×10^6) were assayed in tubes. The cells were washed once with phosphate-buffered saline (PBS) and incubated, if not stated otherwise, 1 hr at 37°C with mAb in 2 ml of complete culture medium. Cells were washed once with ice-cold PBS and incubated 2 hr at 4°C with complete medium containing 10 mM Hepes (GIBCO), 0.1% sodium azide, and 1 nM ^{125}I-TNF-α. After three washes with ice-cold PBS/0.1% bovine serum albumin (BSA) (PBS/BSA) cells were lysed with 1% SDS and bound ^{125}I was determined in a Gammamatic I counter (Kontron, Zurich). Nonspecific binding, as determined in the presence of 500 nM unlabeled TNF, was subtracted. The results were presented as the mean of triplicate assays.

Flow Cytometric Analysis. Adherent cells (HEp-2) were detached with EDTA (GIBCO) and treated analogously to suspension cells 1 hr at 0°C with biotin–mAb at 10 μg/ml in PBS/BSA. Cells were washed once with PBS/BSA, stained with streptavidin–phycoerythrin (Southern Biotechnology, Birmingham, AL) and analyzed ungated by a FACScan flow cytometer (Becton Dickinson).

SDS/PAGE, Immunologic Blotting, Ligand Blotting, and Immunoprecipitation. Specimen enriched for TNF-binding proteins were separated by SDS/PAGE in Mini-Gel boxes (Bio-Rad) with the buffer system of Laemmli (21). Proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Millipore). After blocking free binding sites with 1% defatted milk powder in 50 mM Tris·HCl, pH 7.4/140 mM NaCl/5 mM EDTA/0.02% sodium azide for 1 hr, the membranes were incubated with ^{125}I-TNF-α in the same buffer for 12 hr or with hybridoma supernates followed by ^{125}I-labeled RamIg. The membranes were washed and autoradiographed on x-ray films (XAR-5, Kodak) with intensifier screens (Chronex Li Plus, DuPont). For immunoprecipitation 5×10^6 cells were surface labeled with ^{125}I and lactoperoxidase and lysed in 1% Triton X-100 (Pierce) in 50 mM Tris·HCl, pH 7.4/140 mM NaCl/2 mM EDTA with protease inhibitors. The lysate was centrifuged 10 min at $10^5 \times g$ and precleared 30 min with fixed *Staphylococcus aureus* (Pansorbin, Calbiochem) coated with RamIg. The lysate was treated for 2 hr with mAb at 50 μg/ml and then with RamIg-coated *S. aureus* as before. The precipitate was collected, washed, and analyzed by SDS/PAGE and autoradiography.

RESULTS

Antibodies. The htr and utr series of antibodies were prepared by using purified TNF-binding protein isolated from two different sources as immunogens. The htr antibodies (htr-1 to -9) were obtained with immunogen purified from HL-60 cells, which had $\approx$5000 TNF-binding sites per cell as calculated on the basis of monomeric TNF-α. These antibodies belonged to the IgM (htr-1 and htr-2), to the IgG2b (htr-6 and htr-7), or to the IgG1 subclasses. The utr antibodies (utr-1 to -15) were isolated from a fusion obtained after immunization of a mouse with TNF-binding protein purified from U-937/T cells that expressed $\approx$20,000 high-affinity TNF-binding sites per cell. All utr antibodies were found to be of the IgG1 isotype. The HL-60 and U-937 immunogens had both been subjected to fractionation by HPLC that separated two TNF-binding activities present in both preparations. In each case only the fractions containing the highest TNF-binding activity were used for immunization. This explains the exclusive specificity of either set of antibodies, in spite of the fact that HL-60 and U-937 cells express both antigens simultaneously (see below).

Effects of htr/utr mAbs on TNF Binding. The antibodies were tested at high concentration (330 nM) in ^{125}I-TNF-α-binding assays on a panel of human and murine cell lines. It was found that htr antibodies inhibited ^{125}I-TNF-α binding on HEp-2, HeLa (Fig. 1), and also MCF7 cells (data not shown) but only partially inhibited ^{125}I-TNF-α binding on HL-60 and U-937 cells. In contrast, the antibodies utr-1 and -2 had no effect on the ^{125}I-TNF-α binding on HEp-2 and

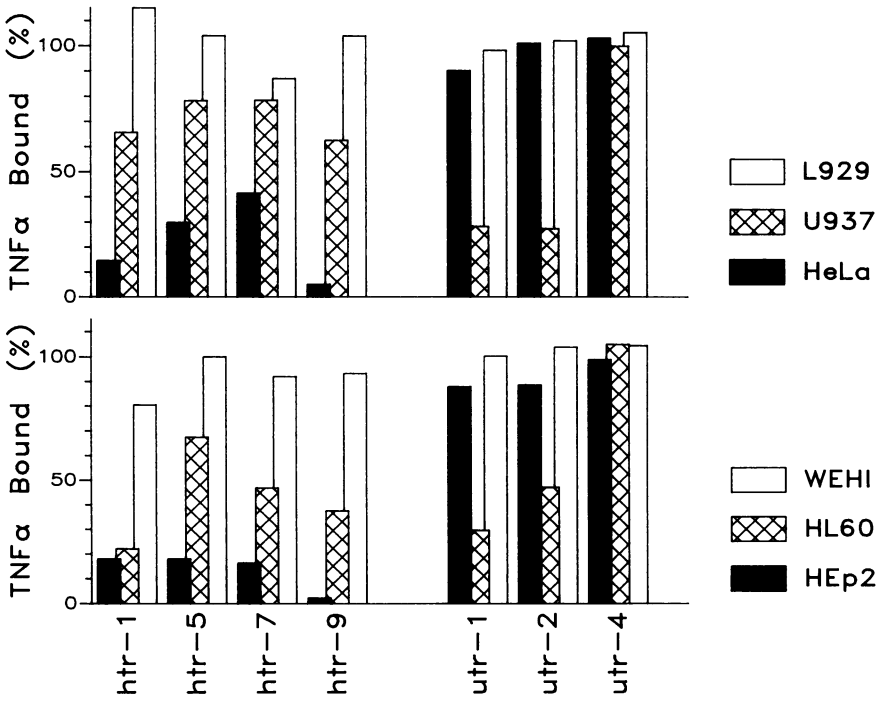

FIG. 1. Effect of mAbs on ^{125}I-TNF-α binding to cell lines. (*Upper*) HeLa, U-937, and L929 cells. (*Lower*) HEp-2, HL-60, and WEHI 164 cells. Cells were incubated with mAb at 50 μg/ml, washed, and incubated with 1 nM ^{125}I-TNF-α, as described. Bound ^{125}I-TNF is expressed as percent of control cells incubated without antibody.

HeLa cells but again partially inhibited ^{125}I-TNF-α binding on HL-60 and U-937 cells. Notably none of these antibodies interfered with ^{125}I-TNF-α binding to the murine cell lines WEHI 164 and L929 (Fig. 1). The antibodies htr-2, -3, -4, and -8 (data not shown) had reactivities similar to htr-1, -5, -7, and -9 (Fig. 1). mAb utr-3 (data not shown) reacted like the utr-1 and utr-2 antibodies, whereas utr-4 (Fig. 1) as well as utr-5 to -15 (data not shown), which were selected only by immunologic blotting, did not inhibit ^{125}I-TNF-α binding. In a different type of assay the cells were incubated simultaneously with antibodies and ^{125}I-TNF-α at 4°C and azide was added to prevent antibody-induced internalization of the antigen. Fig. 2 shows a similar pattern of TNF-binding inhibition on HEp-2 and U-937 cells under these conditions to that of Fig. 1. Binding of ^{125}I-TNF-β and ^{125}I-TNF-α was inhibited by the antibodies to an equivalent extent (Fig. 2).

The data presented in Figs. 1 and 2 suggest that HL-60 and U-937 cells express two different TNF-binding sites that are recognized by the htr and the utr antibodies, respectively, whereas HEp-2 cells express only that type of TNF-binding site recognized by the htr antibodies. To confirm the existence of two distinct TNF-binding sites, HL-60 and U-937 cells were simultaneously incubated with mAb htr-9 and mAb utr-1. Fig. 3 shows that the preincubation with both antibodies caused a complete loss of TNF-α-binding sites. The effects of both antibodies were additive over the whole range of antibody concentrations tested. Purified peripheral blood granulocytes and monocytes showed a similar susceptibility for inhibition to ^{125}I-TNF-α binding by mAb htr-9 and utr-1 as HL-60 cells.

Flow Cytometric Analysis. To examine whether the htr and utr antibodies bind to closely linked or independent cell-surface epitopes, HEp-2, HL-60, U-937, and Raji cells were incubated with the biotin-labeled mAbs htr-9 or utr-1, stained with streptavidin–phycoerythrin, and analyzed by flow cytometry (Fig. 4). Raji cells have no detectable TNF-binding sites (15) and were used to assess the nonspecific binding of the reagents. With HEp-2 cells a positive immunofluorescence was found only with mAb htr-9 and was not found with

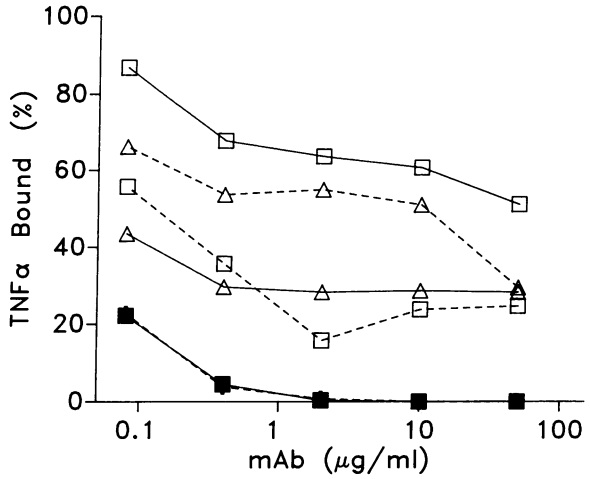

FIG. 3. Complementary inhibition by mAb htr-9 (□) and utr-1 (△) as single antibodies or in a 1:1 mixture of both mAbs (■). ^{125}I-TNF-α binding to HL-60 (- - -) and U-937 (—) is expressed as percent of control cells incubated without antibody. Concentration of individual htr-9 and utr-1 mAbs in the mixture is indicated on the abscissa.

mAb utr-1, whereas HL-60 and U-937 cells bound both antibodies. The staining intensities with both antibodies were reduced to background level when TNF-α was present in excess. Ten other antibodies of the utr series gave a similar surface fluorescence on U-937 cells as mAb utr-1, but only the staining by utr-2 and -3 was blocked by TNF-α (data not shown). We found that the staining intensities of HL-60 and U-937 cells obtained with either biotinylated htr-9 or utr-1 were not reduced by coincubation with an excess of the converse unlabeled antibodies (Fig. 4 *a* and *b*).

Biochemical Characterization of the htr-9 and utr-1 mAb Antigens. To determine the molecular mass of the htr- and utr-mAb antigens, the TNF-binding proteins were enriched from cell lysates by TNF-α affinity chromatography, separated by SDS/PAGE, transferred to filter membranes, and

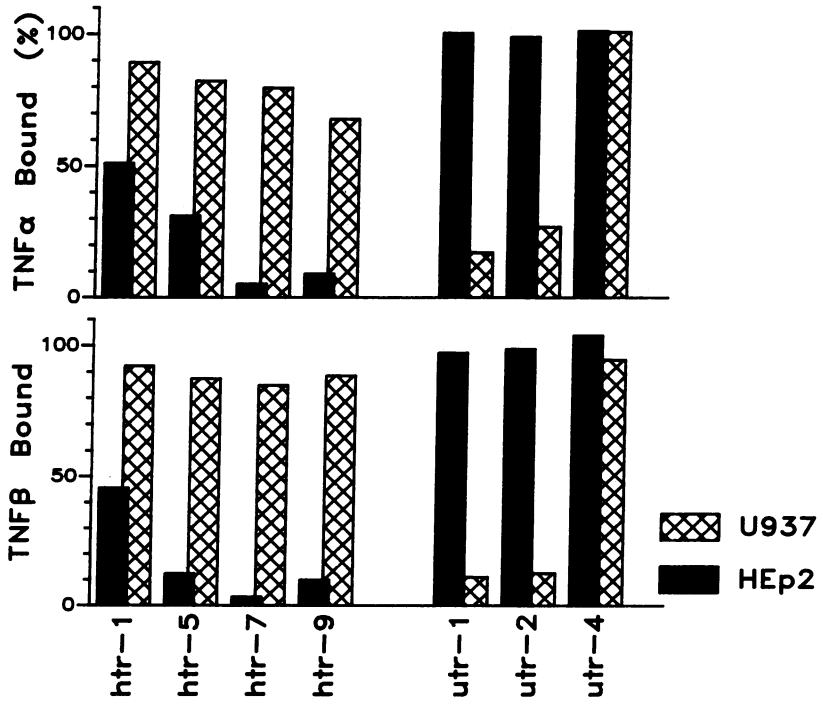

FIG. 2. Comparison of TNF-α and TNF-β binding inhibition on HEp-2 and U-937 cells. Cells were incubated for 2 hr with mAb at 50 μg/ml together with 1 nM ^{125}I-TNF-α (*Upper*) or 1 nM ^{125}I-TNF-β (*Lower*) at 4°C in complete medium with 0.1% sodium azide. Bound ^{125}I label is expressed as percent of control cells without antibody.

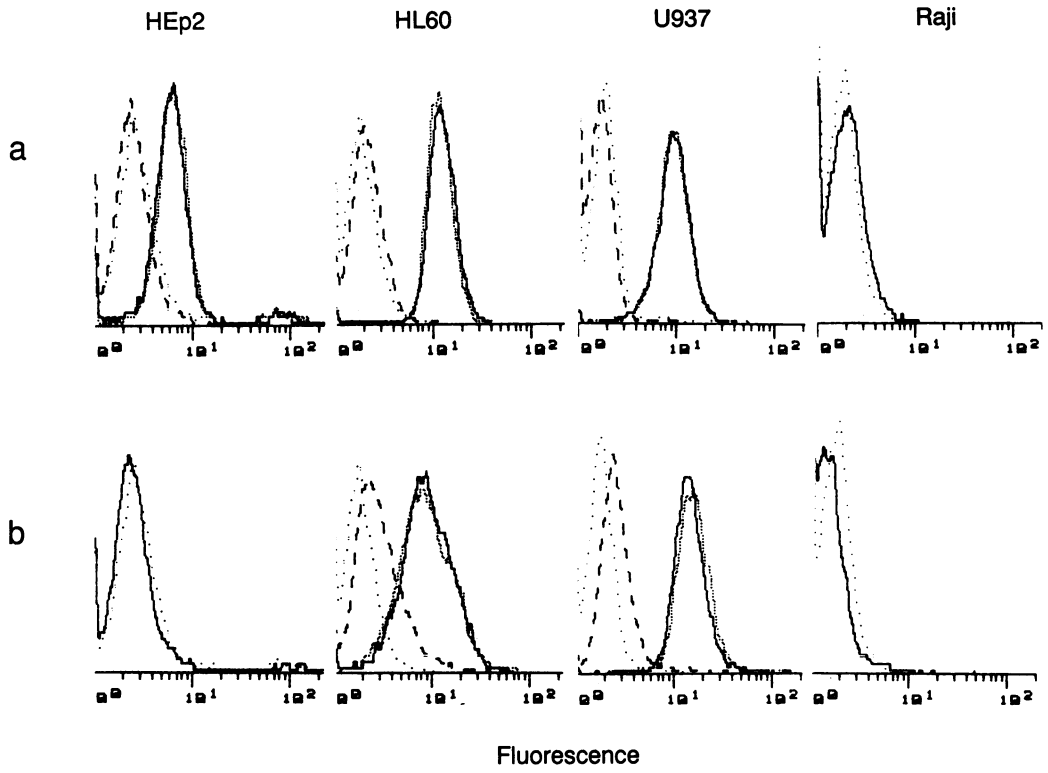

FIG. 4. Flow cytometric analyses on HEp-2, HL-60, U-937, and Raji cells. Relative cell numbers are indicated on the ordinates. Fluorescence obtained with an irrelevant biotinylated control mAb (· · ·). (*a*) Fluorescence with biotin–htr-9 without TNF-α (—) and with TNF-α at 60 μg/ml (- - -) or mAb utr-1 at 100 μg/ml (···). (*b*) Fluorescence with biotin–utr-1 without addition of TNF-α (—) and with TNF-α at 60 μg/ml (- - -) or mAb htr-9 at 100 μg/ml (···).

probed with either ^{125}I-TNF-α (Fig. 5*A*), mAb utr-1 and then ^{125}I-labeled RamIg (Fig. 5*B*), or mAb htr-9 and then ^{125}I-labeled RamIg (Fig. 5*C*). Two major types of TNF-binding proteins with apparent molecular mass of 75/65 kDa (type A) and 55/50 kDa (type B) were identified in the lysate of HL-60 cells, whereas predominantly type-A TNF-binding protein was found in the lysate from U-937/T cells and type-B TNF-binding protein in the lysate of HEp-2 cells. Prolonged exposure of the autoradiograms did not reveal any type-A TNF-binding protein in HEp-2 cells but did show a minor amount of type-B TNF-binding protein in U-937/T cells. The binding of ^{125}I-TNF-α and the antibodies was abolished in the presence of excess unlabeled TNF-α (data not shown). Type A and B proteins were clearly distinct with regard to reactivities with htr-9 and utr-1 antibodies (Fig. 5 *B* and *C*). Without apparent crossreactivity, the htr-9 antibody bound to type-B TNF-binding protein, whereas the utr-1 antibody bound to type-A protein band exclusively. Reduction with 2-mercaptoethanol abolished the reactivity of type A and B proteins with ^{125}I-TNF-α, as well as with mAbs htr-9 and utr-1, indicating that disulfide bonds are essential for the configuration of the binding site of both molecules (data not shown). The size heterogeneity seen with type A and B TNF-binding proteins (Fig. 5) could be due to degradation or differences in glycosylation. Antibody utr-1 precipitated a single 75-kDa band from surface-labeled HL-60 and U-937 cells but not from HEp-2 cells (Fig. 6). Attempts to immunoprecipitate with mAb htr-9 or the other htr antibodies were unsuccessful.

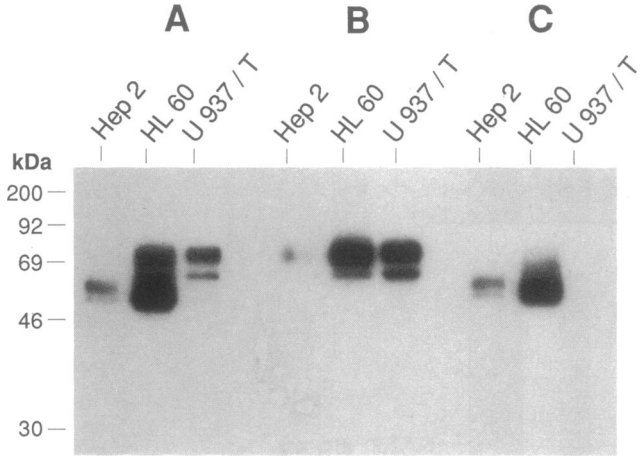

FIG. 5. Ligand and immunologic blotting of TNF-binding proteins from three cell lines. Whole-cell lysates were enriched for TNF-binding proteins by affinity chromatography on TNF-α–Sepharose 4B, separated by SDS/PAGE under nonreducing conditions, and transferred to filter membranes. Blots were incubated with 0.5 nM ^{125}I-TNF-α (*A*), with mAb utr-1 at 30 μg/ml and then with ^{125}I-labeled RamIg (*B*) and with mAb htr-9 at 30 μg/ml and then with ^{125}I-labeled RamIg (*C*). HEp-2 (4.2×10^6), HL-60 (4.5×10^7), and U-937/T (4.1×10^6) cell equivalents per lane were applied. Molecular mass markers are indicated at left.

DISCUSSION

Several laboratories have described a significant size heterogeneity of the TNF receptor. Crosslinking with ^{125}I-TNF-α was reported to produce products of 138, 90, 75, and 54 kDa in MCF7 cells (16), 92 kDa in U-937 cells (15), and 100/75 kDa in HL-60 cells (17). In this study we report that, in agreement with the results by Hohmann *et al.* (17), two discrete types of TNF-binding proteins of 75 and 55 kDa are identified at the cell surface of a variety of cell lines by two sets of noncross-reacting mAbs. HEp-2 and U-937 cells may be considered prototype cells with respect to their reactivity with htr and utr mAbs. HEp-2 cells express predominantly or exclusively 55/50-kDa type-B TNF-binding protein, which is detected by

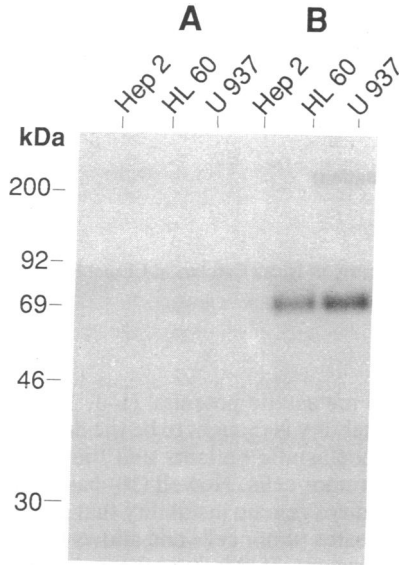

FIG. 6. Immunoprecipitation with mAb htr-9 (*A*) and mAb utr-1 (*B*). Cells (2×10^6) were labeled with ^{125}I and treated as described. SDS/PAGE was done under reducing conditions. Molecular mass markers are indicated at left.

mAb htr-9 and the other htr antibodies. U-937 cells express, in addition to the type-B binding protein, a 75-kda type-A TNF-binding protein that is recognized by mAb utr-1. It is noteworthy that several noninhibiting utr antibodies react in immunologic blot analyses with type-A TNF-binding protein (data not shown), indicating that this protein might be antigenically more diverse than the type-B protein.

TNF-α-binding sites of HL-60 cells have previously been found to have a K_d of 7×10^{-11} M, whereas HEp-2 cells have a K_d of 3×10^{-10} M (17). All inhibition assays in the present study were done at a ligand concentration of 10^{-9} M; we conclude that both type-A and -B TNF-binding proteins have relatively high TNF affinities ($K_d < 10^{-9}$ M). This conclusion supports the view that both molecules are potentially able to function in the signal recognition of TNF and thus might both be considered TNF receptors. Moreover, the existence of two TNF receptors on HL-60 cells may explain the observation of a biphasic dose response of these cells upon induction with TNF (22). The proposition that at least the type-B binding protein is a functional TNF receptor is supported by recent findings that mAbs htr-1 and htr-9, but not mAb htr-5, have a TNF-like activity in assays measuring cytotoxicity, fibroblast growth, interleukin 6 secretion (23), and activation of the transcription factor NF-κB (H.-P. Hohmann, personal communication). By their cytotoxic activity htr-1 and htr-9 are comparable to two other antibodies, anti-fas (24) and anti-APO-1 (25), whose relationship to the TNF receptor is unclear. The antibodies utr-1, utr-2, and utr-3 exhibit no cytotoxic activity (T. Espevik, personal communication).

Stauber and Aggarwal (12) have found that TNF-α and TNF-β compete for the same receptor on U-937 cells but lead to slightly different crosslinking products. We show here that binding of both ligands is inhibited by htr and utr antibodies practically to the same extent (Fig. 2), indicating identical or closely linked binding sites for both ligands. A number of other lympho/cytokine receptors have been reported to consist of two or more polypeptides. The interleukin 2 receptor contains a 55- and a 75-kDa protein that individually and as a complex bind interleukin 2 with different affinities (26). The TNF receptors differ from the interleukin 2 receptor system, however, because there is, so far, no evidence for an intermolecular association between the two TNF receptors. That the pleiotropic nature of TNF relates to the existence of two discrete receptor molecules remains an intriguing hypothesis.

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1. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3666–3670.
2. Helson, L., Green, S., Carswell, E. A. & Old, L. J. (1975) *Nature (London)* **258,** 731–732.
3. Tracey, K. J., Vlassara, H. & Cerami, A. (1989) *Lancet* **i,** 1122–1126.
4. Talmadge, J. E., Phillips, H., Schneider, M., Rowe, T., Pennington, R., Bowersox, O. & Lenz, B. (1988) *Cancer Res.* **48,** 544–550.
5. Kindler, V., Sappino, A.-P., Grau, G. E., Piguet, P.-F. & Vassalli, P. (1989) *Cell* **56,** 731–740.
6. Jacob, C. O. & McDevitt, H. O. (1988) *Nature (London)* **331,** 356–358.
7. Tracey, K. J., Beutler, B., Lowry, S. F., Merryweather, J., Wolpe, S., Milsark, I. W., Hariri, R. J., Fahey, T. J., III, Zentella, A., Albert, J. D., Shires, G. T. & Cerami, A. (1986) *Science* **234,** 470–474.
8. Beutler, B. & Cerami, A. (1987) *N. Engl. J. Med.* **316,** 379–385.
9. Gray, G. E., Fajardo, L. F., Piguet, P.-F., Allet, B., Lambert, P.-H. & Vassalli, P. (1987) *Science* **237,** 1210–1212.
10. Jones, E. Y., Stuart, D. I. & Walker, N. P. C. (1989) *Nature (London)* **338,** 225–228.
11. Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B. & Goeddel, D. V. (1984) *Nature (London)* **312,** 724–729.
12. Stauber, G. B. & Aggarwal, B. B. (1989) *J. Biol. Chem.* **264,** 3573–3576.
13. Rubin, B. Y., Anderson, S. L., Sullivan, S. A., Williamson, B. D., Carswell, E. A. & Old, L. J. (1985) *J. Exp. Med.* **162,** 1099–1104.
14. Munker, R., DiPersio, J. & Koeffler, H. P. (1987) *Blood* **70,** 1730–1734.
15. Scheurich, P., Ucer, U., Kronke, M. & Pfizenmaier, K. (1986) *Int. J. Cancer* **38,** 127–133.
16. Creasey, A. A., Yamamoto, R. & Vitt, C. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3293–3297.
17. Hohmann, H.-P., Remy, R., Brockhaus, M. & van Loon, A. P. G. M. (1989) *J. Biol. Chem.* **264,** 14927–14934.
18. Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80,** 849–857.
19. Updyke, T. V. & Nicolson, G. L. (1984) *J. Immunol. Methods* **73,** 83–95.
20. Stocker, J. (1982) *Res. Discl.* **217,** 155–156.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Ishikura, H., Hori, K. & Bloch, A. (1989) *Blood* **73,** 419–424.
23. Espevik, T., Brockhaus, M., Loetscher, H., Nonstad, U. & Shalaby, R. (1990) *J. Exp. Med.* **171,** 415–426.
24. Yonehara, S., Ishii, A. & Yonehara, M. (1989) *J. Exp. Med.* **169,** 1747–1756.
25. Trauth, B. C., Klas, C., Peters, A. M. J., Matzku, S., Moller, P., Falk, W., Debatin, K.-M. & Krammer, P. H. (1989) *Science* **245,** 301–305.
26. Dukovich, M., Wano, Y., Thuy, L. t. B., Katz, P., Cullen, B. R., Kehrl, J. H. & Greene, W. C. (1987) *Nature (London)* **327,** 518–522.