Title

Enhancement of lymphokine-activated T killer cell tumor necrosis factor receptor mRNA transcription, tumor necrosis factor receptor membrane expression, and tumor necrosis factor/lymphotoxin release by IL-1 beta, IL-4, and IL-6 in vitro.

Journal

The Journal of Immunology, 146(5)

ISSN

0022-1767

Authors

Dett, CA
Gatanaga, M
Ininns, EK
et al.

Publication Date

1991-03-01

DOI

10.4049/jimmunol.146.5.1522

0022-1767/91/1465-1522$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 1522–1526, No. 5, March 1, 1991
Printed in U.S.A.

ENHANCEMENT OF LYMPHOKINE-ACTIVATED T KILLER CELL TUMOR NECROSIS FACTOR RECEPTOR mRNA TRANSCRIPTION, TUMOR NECROSIS FACTOR RECEPTOR MEMBRANE EXPRESSION, AND TUMOR NECROSIS FACTOR/LYMPHOTOXIN RELEASE BY IL-1β, IL-4, AND IL-6 IN VITRO[1]

CARMEN A. DETT,* MAKI GATANAGA,* ELIZABETH K. ININNS,* FABIO CAPPUCCINI,†§ ROBERT S. YAMAMOTO,* GALE A. GRANGER,*‡ AND TETSUYA GATANAGA[2]*

*From the *Department of Molecular Biology & Biochemistry and †Department of Obstetrics & Gynecology, University of California Irvine, Irvine, CA 92717, ‡Memorial Cancer Institute, Long Beach, CA 90801, and §Italian Association for Cancer Research A.I.R.C., Milan, Italy*

Co-culture with IL-2 can induce human T lymphocytes to proliferate and become nongenetically restricted, lymphokine-activated killer (LAK) cells in vitro. Our studies were conducted with long term cultured, human T-LAK cells from peripheral blood, which are 95 to 99% CD3+. We found that proliferating 7- to 10-day human T-LAK cells express TNFR, by using a ^{125}I-TNF binding assay. Additional treatment of these cells with the cytokines IL-1β, IL-4, or IL-6 rapidly up-regulated 55-kDa TNFR mRNA transcription and doubled TNFR membrane expression. Further studies revealed that these cytokines also increased the release of TNF and lymphotoxin (LT). Antibody neutralization studies indicated that IL-1 induces release of both TNF and LT; however, IL-4 and IL-6 induce primarily LT release. These results further support the concept that these cytokines are involved in the regulation of TNF/LT release, TNFR synthesis, and TNFR membrane expression. It is apparent that cytokines and their membrane receptors are involved in the autocrine/paracrine control of T cell proliferation, differentiation, and expression of functional activity after IL-2 stimulation in vitro.

The stimulation of PBMC from normal human donors with PHA for 2 days and co-culture with IL-2 (1) leads to the generation of long term, proliferating, nongenetically restricted T effector cells (2), which we have termed T-LAK[3] cells. Kruse et al. (1) demonstrated that, by day 7, 90–99% of the cells in these cultures are proliferating CD3+ T cells. T-LAK cells are under current investigation for use in experimental intralesional immunotherapy of patients with recurrent brain tumors (1, 3).

TNF and LT are cytokines produced by activated lymphoid cells (4, 5) that cause a wide range of biologic activities in vivo and in vitro. In vivo, TNF is known to be involved in inflammation (6), wasting, and shock (7). In vitro, TNF has direct cytotoxic effects on certain transformed cell lines (8) and is also known to stimulate fibroblast growth (9, 10) and regulation of cell surface Ag expression (11, 12). This cytokine also possesses multifunctional immunoregulatory activities (13), which include activation of NK cells (14) and macrophages (15) and induction of growth and function of B and T lymphocytes in vitro (13, 16).

The biologic effects of TNF/LT, both in vivo and in vitro, are initiated by their first binding to specific cell membrane receptors (17). It has recently been shown that mammalian cells can express one or both of two different TNF/LT receptors, one of 55 kDa (18) and one of 68 to 75 kDa (19). These membrane proteins show some homology in their extracellular domains, but their intracellular domains are totally different in amino acid sequence. The fact that they are two different receptors was not detected by almost all previous investigators. It has been demonstrated that human T-LAK cells express the TNFR but unstimulated T cells do not (13, 20). However, it is not yet known whether these cells express one or both receptors. It has also been shown that TNF and other cytokines are released by human T cells after activation in co-culture with IL-2 in vitro (21). Furthermore, it is known that TNF and IL-2 synergize to augment proliferation of T cells (13) and cytotoxic activity of human T-LAK cells (22, 23). It is becoming apparent that released cytokines and their membrane receptors are involved in T cell proliferation, differentiation, and expression of cytolysis in vitro. We were interested in further examining the role of cytokines and their membrane receptors in the differentiation of T-LAK cells in vitro.

We established the baseline expression of the TNFR on T-LAK cells and then determined the effects of selected cytokines on mRNA transcription of the 55-kDa TNFR, membrane TNFR expression, and TNF/LT release. We found that cytokines IL-1β, IL-4, and IL-6 up-regulate 55-kDa TNFR transcription, TNFR expression, and TNF/LT release in human T-LAK cells in vitro. TNF and LT may be important immunoregulatory molecules in the function and growth of T-LAK cells.

Received for publication September 4, 1990.
Accepted for publication November 27, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by grants from Memorial Cancer Institute of Long Beach Memorial Hospital (Long Beach, CA) and the Cancer Research Coordinating Committee of the University of California.
[2] Please address all correspondence and requests for reprints to Tetsuya Gatanaga, Ph.D., Department of Molecular Biology & Biochemistry, University of California, Irvine, Irvine, CA 92717.
[3] Abbreviations used in this paper: T-LAK, lymphokine-activated killer T cells; LT, lymphotoxin.

MATERIALS AND METHODS

Cells, cytokines, and reagents. L929 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS (GIBCO Laboratories Life Technologies, Inc., Grand Island, NY). Human PBL were cultured in AIM-V (GIBCO) supplemented with 2% FCS. rTNF/LT and rIL-1β were supplied by Genentech Corp. (South San Francisco, CA). rIL-2 was obtained from the Cetus Corp. (Emeryville, CA). rIL-4 and rIL-6 were obtained from Genzyme (Boston, MA). Bolton-Hunter reagent was purchased from DuPont (Wilmington, DE). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Collection of human PBMC. Human PBMC were collected from peripheral venous blood of normal donors, which was defibrinated, diluted with an equal volume of PBS, and separated by density gradient centrifugation on 1.077 Histopaque (Sigma). The PBMC were collected from the interface of the plasma and Histopaque and further washed, to remove the remaining Histopaque, by alternate centrifugation (500 × *g* for 10 min). After the final centrifugation, the cell pellet was resuspended in AIM-V with 2% FCS and the cells were counted using a SPotlite hemacytometer. These cells were 99 to 100% viable, as determined by trypan blue exclusion test.

Generation of T-LAK. Human PBMC were initially cultured in AIM-V with 2% FCS, supplemented with 400 U/ml human rIL-2 and 0.4 μg/ml PHA. The T-LAK cells were cultured by a method similar to that described by Ingram et al. (3), at a cell density of 2.0×10^6 cells/ml, in a T-75 tissue culture flask. After 48 to 72 h at 37°C, a cell sample in trypan blue was counted on a SPotlite hemacytometer and resuspended at 0.5×10^6 viable cells/ml in complete medium, which contained AIM-V with 2% FCS plus 400 U/ml rIL-2. These T-LAK cell cultures were recultured every 48 h in fresh complete medium, at a density of 0.5×10^6 cells/ml.

Flow cytometric analysis. Cell suspensions were analyzed by FACScan (Becton-Dickinson, Mountain View, CA). The FACscan was calibrated with the Autocomp program and optimized for each sample tested. Forward scatter thresholds were set in order to eliminate debris and dead cells. Particles with unusually low forward and side scatter were considered to be nonviable and were not counted. Surface phenotypes on lymphocytes were analyzed by staining the cells for surface Ag with the following mAb: (a) CD3 (pan-T), (b) CD4 (helper-inducer), and (c) CD8 (cytotoxic-suppressor). The mAb were fluorescein conjugated and were obtained from Becton Dickinson Immunocytometry Systems. Cells were incubated with mAb for 30 min on ice and were washed twice with PBS with 0.2% sodium azide, by alternate centrifugation (5 min at 400 × *g*) and resuspension. Cells were fixed in 1.0% paraformaldehyde solution and stored at 4°C until further analysis. Data were expressed as percentage of positive cells for corresponding surface Ag.

L929 cytotoxic assay. T cell culture supernatants were assayed for TNF or LT activity against the L929 mouse fibroblast cell line by the method of Gatanaga et al. (24). Briefly, L929 cells (8×10^4 cells/well), culture supernatant, and actinomycin D (0.2 μg/well) were incubated at 37°C for 18 h in 96-well flat-bottomed microcytotoxicity plates (Corning), in 200 μl/well RPMI 1640 with 10% FCS. After incubation, the supernatants were aspirated and the plate was stained with 1% crystal violet for 5 min at room temperature. Excess dye was rinsed from the plate with water, and the plate was allowed to dry and was solubilized with 100 μl of acidified methanol (100 mM HCl in methanol). Absorbance was measured at 600 nm. The amount of TNF and LT bioactivity was determined by comparing activity with a standard curve made with rTNF-α and rLT (Genentech). Antibody neutralization studies were carried out in the same manner; however, antiserum was added in conjunction with the culture supernatants. Immune polyclonal antisera were generated against human rLT and rTNF, in NZW rabbits, by the method of Vaitukaitis et al. (25).

Iodination of rTNF and binding assay. rTNF (200 μg) was equilibrated in PBS by exhaustive dialysis against PBS, in 6000–8000 m.w. cut-off Spectra-por dialysis tubing (Spectrum Medical Inc., Los Angeles, CA). After the dialysis, rTNF was concentrated using a Speed Vac concentrator (Savant, NY). The final concentration of rTNF was 2 mg/ml. The rTNF iodination procedure was performed as described by Bolton and Hunter (26). rTNF (100 μg) in 20 μl of 0.1 M sodium borate buffer, pH 8.5, was added to the dried iodinated ester ^{125}I-Bolton-Hunter reagent and the reaction mixture containing Bolton-Hunter reagent and ^{125}I (DuPont). Free iodine was removed by gel filtration over a NAP-5 column (Pharmacia LKB Biotechnology, Inc., Piscatway, NJ) with PBS containing 5 mg of gelatin. Fractions of 150 μl were collected, and 1-μl aliquots were assayed by Clinigamma counter (Pharmacia). Peak fractions were pooled, and the specific activity was determined to be 5400 cpm/ng. The labeled material was stored at 4°C and used within 1 month.

Binding studies and Scatchard analyses. One million cells were incubated with binding buffer (RPMI 1640 with 10% FCS) in 24-well plates and then incubated with 0.5 to 30 ng of ^{125}I-TNF, in the presence or absence of a 100-fold excess of unlabeled TNF. After a 3- to 4-h incubation at 4°C, binding buffer was removed and the cells were washed two to four times with ice-cold PBS (27). Cells were solubilized in 1 ml of 0.3 N NaOH and radioactivity was determined by Clinigamma counter (Pharmacia). Cells were incubated at 37°C for 4 h before the binding assay when the effects of IL-1β (50 ng/ml), IL-4 (20 ng/ml), and IL-6 (50 ng/ml) on rTNF binding were examined.

Specific oligonucleotide probe for the 55-kDa TNFR. The oligonucleotide 60-mer for the human TNFR probe 5′-GCCACACACGGTGTCCCGGTCCACTGTGCAAGAAGAGATCTCCACCTGACCCATTTCCT-3′ (amino acids 67 to 86) (18) was synthesized by using a Biosearch 8600 DNA synthesizer. The oligonucleotide probe was end-labeled by [γ-^{32}P]ATP (New England Nuclear, Boston, MA), using T_4 polynucleotide kinase (28).

RNA isolation and Northern blot analysis. Total cellular RNA was isolated from T-LAK cells by the guanidinium isothiocyanate/cesium chloride method and was quantitated spectrophotometrically as previously described (29). Integrity of the RNA was confirmed with ethidium bromide (0.5 mg/ml). RNA (5 to 10 μg) was electrophoresed in 2.2 M formaldehyde-1.2% agarose gels, transferred to nitrocellulose filters (Schleicher & Schuell, Keene, NH), and baked at 80°C for 2 h, as previously described (30). The filters were hybridized with ^{32}P-labeled TNFR-specific synthetic probe. After hybridization, blots were washed at 25°C with 2× SSC, 0.1% SDS, for 30 min, and then with 0.1× SSC, 0.1% SDS, for 30 min. The blots were then exposed to X-Omat XAR-5 (Kodak Co., Rochester, NY) X-ray film at −70°C.

RESULTS

Expression of T lymphocyte surface phenotypic markers by 7–10-day T-LAK cells. T-LAK cells were generated as described in *Materials and Methods* and were subjected to phenotypic analysis on day 7 and day 10 of culture. Shown in Figure 1 are the data obtained from phenotyping of one individual donor; however, these results are representative of data from all normal individuals (10 subjects) examined. These data reveal that CD3$^+$ T cells dominate (90–95%) the culture by days 7 to 10 and, although not shown, remain the predominant cell type throughout the culture period (days 15 to 21). The ratio of CD4$^+$ to CD8$^+$ cells in these cultures from a single donor remained relatively constant; however, they vary from individual donor to donor.

Capacity of IL-1β, IL-4, and IL-6 to induce the release of TNF/LT by T-LAK cells in vitro. T-LAK cells were incubated with the cytokines IL-1β, IL-4, or IL-6 (in addition to IL-2) for 24 h, and then cell-free supernatant cytotoxic activity was measured in the L929 assay, as described in *Materials and Methods*. Supernatants from cells incubated with cytokines for a period of 4 h did not

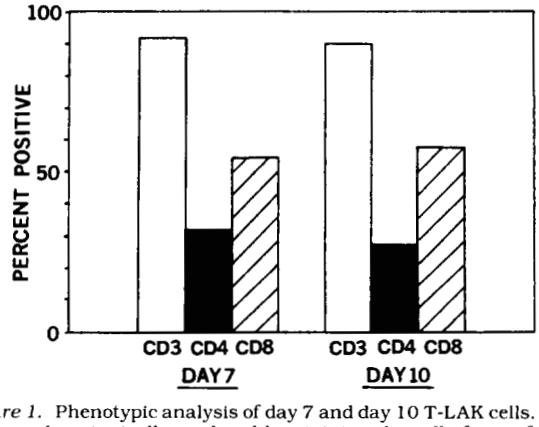


Figure 1. Phenotypic analysis of day 7 and day 10 T-LAK cells. T-LAK cells were phenotypically analyzed by staining the cells for surface Ag, with mAb to CD3 (pan T), CD4 (helper-inducer), and CD8 (cytotoxic-suppressor). Data are expressed as percentage of positive cells for the corresponding surface Ag.

show any cytolytic activity. Figure 2 shows that supernatants from T-LAK cells incubated with these cytokines for 24 h produce cytotoxic activity against L929 cells. Supernatants from T-LAK cells incubated with IL-4 produced the highest amount of cytotoxic activity, followed by IL-6 and IL-1β. However, control T-LAK cells incubated with IL-2 alone produced much less. Additional studies revealed that the cytotoxic activity of all T-LAK supernatants was blocked 75 to 95% by the anti-LT antiserum, and 45% of the cytolytic activity produced by IL-2- and IL-1β-stimulated cells was neutralized by anti-TNF antiserum. Thus, the majority of the cytolytic activity of these supernatants was due to LT and not TNF.

Effects of IL-1β, IL-4, and IL-6 on the expression of the TNFR on T-LAK cells. The expression of TNFR on human T-LAK cells (days 7 to 17) was examined in a ^{125}I-TNF-binding assay, as described in *Materials and Methods*. The expression of TNFR was examined at 4 and 24 h after incubation with the cytokines IL-1β, IL-4, and IL-6 (in addition to IL-2). Results from one experiment are shown in Figure 3, *A* and *B*. Control T-LAK cells (cocultured with IL-2 alone) expressed 674 receptors on day 10 of culture (Fig. 3*B*); however, when these cells were incubated with IL-1β, IL-4, or IL-6 (in addition to IL-2) for 4 h, an increase of 1341, 1241, and 1172, respectively, in receptor number (74 to 99%) was detected. The apparent K_d values were 1.8×10^{-10} M for IL-2, 1.3×10^{-10} M for IL-1β, 1.4×10^{-10} M, for IL-4, and 1.1×10^{-10} M for IL-6, as determined by Scatchard analyses. There were clearly no significant changes in receptor affinity. When T-LAK cells were treated with these cytokines for 24 h, no further up-regulation of TNFR was detected (data not shown). A slight variation in receptor number was noted in experiments conducted on cells from different donors; however, results from multiple experiments were essentially identical.

Effects of IL-1β, IL-4, and IL-6 on 55-kDa TNFR mRNA expression by T-LAK cells. Human T-LAK cells were exposed to the different cytokines and then tested for 55-kDa TNFR mRNA expression by Northern blot analysis, as described in *Materials and Methods*. Results in Figure 4*A* demonstrate that T-LAK cells cultured with IL-2 expressed mRNA for the 55-kDa TNF/LTR. However, after a 4-h incubation with IL-1β, IL-4, or IL-6 (in addition to IL-2), an up-regulation in 55-kDa mRNA was evident. Duplicate samples were run on the same gels to permit quantitation of the amount of RNA loaded/lane. The gel was cut and stained with ethidium bromide. Results are shown in Figure 4*B*. The samples all produced 28S ribosomal RNA bands in similar regions and of similar intensity.

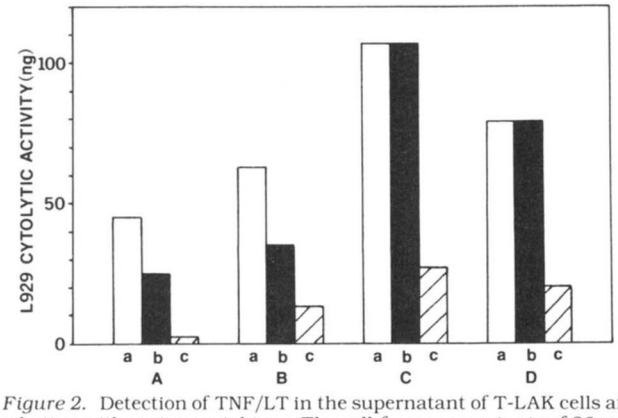
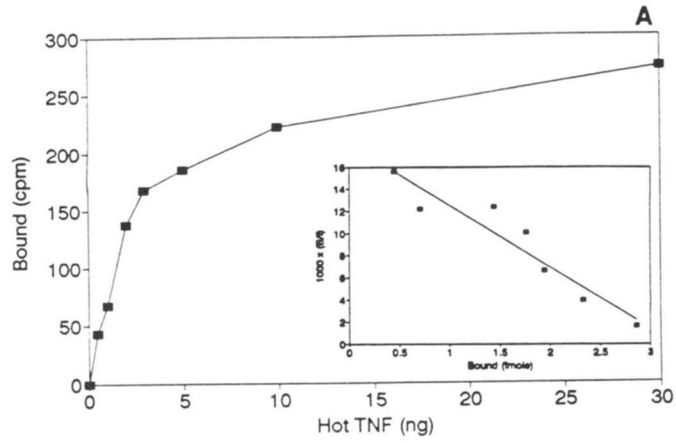


Figure 2. Detection of TNF/LT in the supernatant of T-LAK cells after incubation with various cytokines. The cell-free supernatants of 20×10^6 T-LAK cells, incubated for 24 h in 10 ml of AIM-V with 2% FCS and various cytokines, were tested against L929 cells, as described in *Materials and Methods*. Data are expressed in ng of TNF/LT activity in 10 ml of culture supernatants. *A*, IL-2 only (400 U/ml); *B*, IL-2 plus IL-1β (50 ng/ml); *C*, IL-2 plus IL-4 (20 ng/ml); *D*, IL-2 plus IL-6 (50 ng/ml). *a*, Supernatant alone; *b*, supernatant plus rabbit anti-TNF; *c*, supernatant plus rabbit anti-LT.



B

CYTOKINE	INCREASE IN RECEPTOR # (%)
CONTROL	0
IL-1	99
IL-4	84
IL-6	74

Figure 3. Effects of IL-1β, IL-4, and IL-6 on the expression of TNF membrane receptors on T-LAK cells in vitro. The indicated amounts of ^{125}I-rTNF were added to 10^6 normal human T-LAK cells at 4°C for 3 h. Specific binding represents total bound radioactivity minus that bound in the presence of a 100-fold excess of unlabeled TNF. Data shown are expressed as counts bound/cell. *A*, The results of the 4-h control. *Inset*, Scatchard analysis of the equilibrium binding data. *B/F*, bound/free; *B*, The percentage of increase in receptor number after 4-h cytokine incubation. Percentage increase = (incubated sample TNFR number − control TNFR number)/(control TNF-R number × 100.

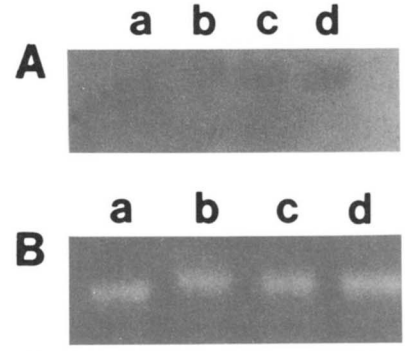


Figure 4. Level of TNFR mRNA expression in T-LAK cells after incubation with various cytokines. T-LAK cells (50×10^6) were incubated with IL-2 plus IL-1β (50 ng/ml) (*a*), IL-2 only (400 U/ml) (*b*), IL-2 plus IL-6 (50 ng/ml) (*c*), or IL-2 plus IL-4 (20 ng/ml) (*d*), for 4 h in 10 ml of AIM-V with 2% FCS. Total RNA was extracted as described in *Materials and Methods*. *A*, Representative Northern blot; 10 μg of total RNA for each sample were analyzed. The filters were sequentially hybridized with the ^{32}P-labeled oligonucleotide probe for the human TNFR. *B*, Representative Northern gel; 2 μg of total RNA for each sample were analyzed in a gel stained with ethidium bromide, to ensure the quantity of samples loaded.

DISCUSSION

The generation of T-lymphokine-activated killer cells, expressing T cell phenotypes, from human peripheral blood has been described (31). These non-MHC-restricted T-LAK cells exhibit a broad range of cytotoxic activity against panels of target cells in vitro (2). Whereas IL-2 stimulates proliferation of T-LAK cells, other cytokines, such as TNF, have been shown to be involved in the expression of cytolytic activity in vitro (22, 23). Little is known, however, with regard to the effects of various cytokines on the control of TNF/LT release and membrane receptor regulation.

Much effort is now being aimed at defining the function and regulatory activity of cytokine receptors on T lymphocytes. Others have suggested that up-regulation of TNF membrane receptors is involved in the autocrine/paracrine pathway operative after IL-2 stimulation and that interaction of TNF/LT with these receptors is important in T cell proliferation, differentiation, and expression of functional ability (13, 20). Fresh unstimulated PBL do not express TNFR of any type (13, 20). Although it is not yet apparent which class of receptors are expressed, Owen-Shaub et al. (20) demonstrated that lymphocyte TNFR expression is regulated by IL-2 concentration in vitro. However, although the presence of surface TNFR on activated T-lymphocytes has been established, the mechanisms of receptor regulation are unknown.

It has been shown that various cytokines can influence both proliferation and expression of T cell function. IL-1β, IL-4, and IL-6 have been shown to induce human and murine thymocyte proliferation (32–34). Suda et al. (34) reported that IL-4 induced murine T cell proliferation and IL-1β together with IL-6 enhanced proliferation induced by IL-2. It is also known that activated T cells have the capacity to produce IL-2, IL-4, IL-6, and TNF/LT and that these cytokines are involved in an autocrine loop of T cell expression of cytotoxicity and proliferation in vitro (22, 23, 35).

We report here that TNFR number, 55-kDa mRNA expression, and TNF/LT release can be up-regulated, in response to IL-1β, IL-4, and IL-6 incubation, on human T-LAK cells co-cultured with IL-2. Although these studies do not distinguish whether up-regulation of receptors involves 55- or 75-kDa receptors, or both, we noted dramatic up-regulation within 4 h. In fact, by Scatchard analysis the number of sites/cell for IL-1β-, IL-4-, and IL-6-treated cells increased almost 2-fold. In addition, we found that 4-h incubation of day 10 T-LAK with IL-1β, IL-4, and IL-6 resulted in an up-regulation of 55-kDa TNFR mRNA expression. This finding agrees with the Scatchard analysis data, in that TNFR mRNA enhancement correlates with an increase in TNFR membrane expression. It is interesting that opposite results are observed with stimulated human monocyte-like cell lines; namely, LPS, PMA, and IL-1β rapidly down-regulate TNFR membrane expression. It is also interesting to note that T-LAK cell TNF/LT release was not apparent at 4 h but was up-regulated by each cytokine after 24 h. IL-4 was the most effective in up-regulating TNF/LT release. Both IL-2 and IL-1β induced about equal amounts of TNF and LT; however, IL-4 and IL-6 induced predominantly LT. It is evident and perhaps slightly significant that there is some selective induction of LT/TNF by these cytokines.

The evidence presented here further supports the concept that IL-2 is sufficient to induce T cell proliferation but is not the sole cytokine involved in the regulation of TNF/LT release or TNFR synthesis and membrane expression. IL-1β, IL-4, and IL-6 can also be involved in the up-regulation of the TNFR at the level of both mRNA transcription for the 55-kDa receptors and membrane expression. In the latter case, it is not yet clear whether this is selective expression of 55-kDa or 75-kDa receptors or up-regulation of both. It is not yet clear whether the T-LAK cell can express the 75-kDa receptor. The exact mechanism involved in the immunoregulation and differentiation of T-LAK effector cells and the specific role of the TNFR is unclear; however it is apparent that cytokines are important in regulating the magnitude of the immune response.

REFERENCES

1. **Kruse, C. A., D. H. Mitchell, K. O. Lillehei, S. D. Johnson, E. L. McCleary, G. E. Moore, S. Waldrop, and W. Mierau.** 1989. Interleukin-2-activated lymphocytes from brain tumor patients. *Cancer 64:61.*
2. **Grimm, E. A., A. Mazumder, H. Z. Zhang, and S. A. Rosenberg.** 1982. Lymphokine-activated-killer cell phenomenon: lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J. Exp. Med. 155:1823.*
3. **Ingram, M., S. Jacques, D. B. Freshwater, G. B. Techy, S. C. Hunter, and J. T. Helsper.** 1987. Salvage immunotherapy of malignant glioma. *Arch. Surg. 122:1483.*
4. **Old, L. J.** 1985. Tumor necrosis factor (TNF). *Science 230:630.*
5. **Beutler, B., and A. Cerami.** 1988. The history, properties, and biological effects of cachectin. *Biochemistry 27:7575.*
6. **Averbook, B. J., R. S. Yamamoto, T. R. Ulich, E. W. Jeffes, I. Masunaka, and G. A. Granger.** 1987. Purified native and recombinant human alpha lymphotoxin (tumor necrosis factor, TNF-beta) induces inflammatory reactions in normal skin. *J. Clin. Immunol. 7:333.*
7. **Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R. J. Hariri, T. J. Fahey, A. Zentella, and J. D. Albert.** 1986. Shock and tissue injury induced by recombinant human cachectin. *Science 234:470.*
8. **Ruggiero, V., K. Latham, and C. Baglioni.** 1987. Cytostatic and cytotoxic activity of tumor necrosis factor on human cancer cells. *J. Immunol. 138:2711.*
9. **Sugarman, B. J., B. B. Aggarwal, P. E. Hass, I. S. Figari, M. A. Palladino, and H. M. Sheppard.** 1985. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. *Science 230:943.*
10. **Palombella, V. J., D. Henriksen-DeStefano, C. Swenson, R. Feinman, M. Hirai, and M. Tsujimoto.** 1986. Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. *J. Exp. Med. 163:632.*
11. **Collins, T., L. A. Lapierre, W. Fiers, J. L. Strominger, and J. S. Pober.** 1986. Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblasts in vitro. *Proc. Natl. Acad. Sci. USA 83:446.*
12. **Pfizenmaier, K., P. Scheurich, C. Schlüter, and M. Krönke.** 1987. Tumor necrosis factor enhances HLA-ABC and HLA-DR gene expression in human tumor cells. *J. Immunol. 138:975.*
13. **Scheurich, P., B. Thoma, U. Ücer, and K. Pfizenmaier.** 1987. Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-α: induction of TNF receptors on human T cells and TNF-α-mediated enhancement of T cell responses. *J. Immunol. 138:1786.*
14. **Ostensen, M. E., D. L. Thiele, and P. E. Lipsky.** 1987. Tumor necrosis factor-α enhances cytolytic activity of human natural killer cells. *J. Immunol. 138:4185.*
15. **Phillip, R., and L. B. Epstein.** 1986. Tumor necrosis factor as an immunomodulator and mediator of monocyte cytotoxicity induced by itself, gamma interferon, and interleukin 1. *Nature 323:86.*
16. **Kehrl, J. H., M. Alvarez-Mon, G. A. Delsing, and A. S. Fauci.** 1987. Lymphotoxin is an important T cell-derived growth factor for human B cells. *Science 238:1144.*
17. **Watanabe, N., Y. Niitsu, N. Yamauchi, H. Umeno, H. Sone, H. Neda, and I. Urushizaki.** 1988. Antitumor synergism between recombinant human tumor necrosis factor and recombinant human interferon-γ. *J. Biol. Response Modif. 7:24.*
18. **Schall, T. J., M. Lewis, K. J. Koller, A. Lee, G. C. Rice, G. H. W.**

Wong, T. Gatanaga, G. A. Granger, R. Lentz, H. Raab, W. J. Kohr, and D. V. Goeddel. 1990. Molecular cloning and expression of a receptor for human tumor necrosis factor. *Cell 61:361.*

19. **Smith, C. A., T. Davis, D. Anderson, L. Solam, M. P. Beckmann, R. Jerzy, S. K. Dower, D. Cosman, and R. D. Goodwin.** 1990. A receptor for human tumor necrosis factor defines an unusual family of cellular and viral proteins. *Science 248:1019.*
20. **Owen-Schaub, L. B., W. L. Crump III, G. I. Morin, and E. A. Grimm.** 1989. Regulation of lymphocyte tumor necrosis factor receptors by IL-2. *J. Immunol. 143:2236.*
21. **Nedwin, G. E., L. P. Svedersky, T. S. Bringman, M. A. Palladino, Jr., and D. V. Goeddel.** 1985. Effect of interleukin-2, interferon-gamma, and mitogens on the production of tumor necrosis factors α and β. *J. Immunol. 135:2492.*
22. **Owen-Schaub, L. B., J. U. Gutterman, and E. A. Grimm.** 1988. Synergy of tumor necrosis factor and interleukin 2 in the activation of human cytotoxic lymphocytes: effect of tumor necrosis factor alpha and interleukin 2 in the generation of human lymphokine-activated killer cell cytotoxicity. *Cancer Res. 48:788.*
23. **Yang, S. C., L. Owen-Schaub, E. A. Grimm, and J. A. Roth.** 1989. Induction of lymphokine-activated killer cytotoxicity with interleukin-2 and tumor necrosis factor-α against primary lung cancer targets. *Cancer Immunol. Immunother. 29:193.*
24. **Gatanaga, T., T. Takahashi, M. Yamazaki, D. Mizuno, and S. Abe.** 1985. Combination antitumor therapy with rabbit tumor necrosis factor and chemo- and immuno-therapeutic agents against murine tumors. *Jpn. J. Cancer Res. 76:631.*
25. **Vaitukaitis, J., J. B. Robbins, E. Nieshlag, and G. T. Ross.** 1971. A method for producing specific antisera with small doses of immunogen. *J. Clin. Endocrinol. 33:988.*
26. **Bolton, A. E., and W. M. Hunter.** 1973. Labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent: application to the radioimmunoassay. *Biochem. J. 133:529.*
27. **Shalaby, M. R., M. A. Palladino, S. E. Hirabayashi, T. F. Eessalu, G. D. Lewis, H. M. Shepard, and B. B. Aggarwal.** 1987. Receptor binding and activation of polymorphonuclear neutrophils by tumor necrosis factor-alpha. *J. Leukocyte Biol. 41:196.*
28. **Chacaonas, G., and J. H. Sande.** 1980. 5′ ^{32}P labeling of RNA and DNA restriction fragment. *Methods Enzymol. 65:75.*
29. **Chirgwin, J. J., A. E. Przbyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry 18:5294.*
30. **Lehrach, H., D. Diamond, J. M. Wonzny, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions. *Biochemistry 16:4743.*
31. **Ferrini, S., S. Meischer, M. R. Zocchi, V. Von Flieder, and A. Moretta.** 1987. Phenotypic and functional characterization of recombinant interleukin-2 (rIL-2) induced activated killer cells: analysis at the population and clonal levels. *J. Immunol. 138:1297.*
32. **Ranges, G. E., A. Zlotnik, T. Espevik, C. A. Dinarello, A. Cerami, and M. P. Palladino.** 1988. Tumor necrosis factor alpha/cachectin is a growth factor for thymocytes: synergistic interactions with other cytokines. *J. Exp. Med. 167:1472.*
33. **Hodgkin, P., M. Bond, A. O'Garra, G. Frank, F. Lee, R. Coffman, A. Zlotnik, and M. Howard.** 1988. Identification of interleukin-6 as a T cell derived factor which enhances the proliferative response of thymocytes to IL-4/PMA. *J. Immunol. 141:151.*
34. **Suda, T., R. Murray, C. Guidos, and A. Zlotnik.** 1990. Growth-promoting activity of IL-1α, IL-6, and tumor necrosis factor-α in combination with IL-2, IL-4, or IL-7 on murine thymocytes: differential effects on CD4/CD8 subsets and on CD3$^+$/CD3$^-$ double-negative thymocytes. *J. Immunol. 144:3039.*
35. **Damle, N. K., and L. V. Doyle.** 1989. Distinct regulatory effects of IL-4 and TNF-α during CD3-dependent and CD3-independent initiation of human T-cell activation. *Lymphokine Res. 8:85.*