Binding and Biological Effects of Tumor Necrosis Factor Alpha on Cultured Human Neonatal Foreskin Keratinocytes

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Abstract

Tumor necrosis factor alpha (TNF alpha) localizes to the epidermis when injected in vivo, but its role in the skin has heretofore not been evaluated. As a first approach to assessing the role of TNF alpha in the skin, we evaluated the binding and biological effects of TNF alpha on human neonatal foreskin keratinocytes maintained in culture. We found that TNF alpha at 0.3–1.0 nM inhibited proliferation of keratinocytes in a reversible fashion as demonstrated by a reduction in total DNA content and clonal growth. The antiproliferative effects were most marked when TNF alpha was added in the preconfluent stages of cell growth. Accompanying this antiproliferative effect was a stimulation by TNF alpha of differentiation of keratinocytes as indicated by the stimulation of cornified envelope formation. Keratinocytes specifically bound TNF alpha, reaching maximal binding in 2 h at 34°C or 8 h at 4°C. Much of the apparent binding at 34°C was due to internalization of the TNF alpha. At 4°C the rate of internalization was much less. Confluent keratinocytes showed a single class of high-affinity receptors with 1,250 receptors/cell and a K_d of 0.28 nM. These data suggest a role for TNF alpha in the growth and differentiation of the epidermis.

Introduction

The epidermis undergoes a continuous process of growth and differentiation as cells progress from the basal proliferating layer to the stratum corneum which is eventually sloughed off. A similar process is observed in vitro with cultured epidermal cells or keratinocytes (1–3), making these cells a useful model for the study of growth and differentiation. Cytokines such as epidermal growth factor (4), transforming growth factors (5), and IFN (6), as well as retinoids (7), vitamin D metabolites (8, 9), calcium (10, 11), and cAMP (12) are all known to alter the growth and differentiation of keratinocytes. Tumor necrosis factor alpha (TNF alpha),[1] a cytokine secreted by stimulated macrophages, is known to bind to and regulate the growth and function of a number of cells in vitro (13–18). A highly significant amount (30%) of TNF alpha localizes to the epidermis after intravenous administration (19), raising the possibility that the keratinocyte is also a target for TNF alpha binding and action. The results to be described in this report indicate that TNF alpha inhibits proliferation but stimulates differentiation of keratinocytes at concentrations equivalent to the K_d of the keratinocyte receptors for TNF alpha.

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1. *Abbreviations used in this paper:* TNF alpha, tumor necrosis factor alpha.

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Methods

Cell culture. Human keratinocytes were isolated from human newborn foreskins by treatment with 0.25% trypsin overnight at 4°C. This procedure is selective for keratinocytes since fibroblasts require collagenase for isolation. The cells were plated onto a feeder monolayer of mitomycin C-treated 3T3 cells in 6-well multiwell plates and grown in DME containing 5% FCS, epidermal growth factor, cholera toxin, hydrocortisone, and antibiotics (20). A small number of 3T3M cells (< 5% of the original number of 3T3 cells plated) remained attached to the dish under the overlaying keratinocytes in preconfluent cultures as determined by differential staining with acridine orange. Such cells are seldom observed after confluence is achieved. First or second passage cells were studied at different time points after plating (0–22 d) in all of these studies. Since the growth rate varied slightly for each keratinocyte culture, the day at which they were deemed confluent varied slightly from experiment to experiment. Each experiment was performed using the same batch of cells and each data point was the mean of triplicate culture dishes.

Effect of TNF alpha on growth of keratinocytes. To determine the effect of TNF alpha on the growth of keratinocytes, different amounts of TNF alpha (0.1–10 nM) were added 24 h after plating and the cells were harvested on day 5 (80% confluent), day 8 (100% confluent), or day 22 (2 wk postconfluent). In other experiments TNF alpha (1.7 nM) was added at different stages of growth, beginning on day 0, 3, 5, 7, or 10. The cells were grown for 12 d. When the medium was changed (every 2 or 3 d) fresh TNF was added to the appropriate cultures. In experiments in which reversibility of TNF alpha was assessed, cells from day 1 were exposed to TNF alpha for 5 d and then cultured in the absence of TNF alpha for up to 14 d. Cells were harvested at different days for DNA assay and compared with cells that received TNF alpha throughout the culture period or with cells that did not receive TNF alpha. For the determinations of DNA, protein, and cornified envelope content, cells were harvested from the plate by scraping into 1 ml of PBS at specified time points and kept frozen until subsequent analysis.

Clonal growth assays were performed in 60-mm dishes containing 5 ml media. Cells were inoculated at a density of 10^4 cells/dish and the indicated amounts of TNF alpha (0.1–10.0 nM) were added 24 h after plating. The plates were incubated at 34°C in a humidified CO_2 incubator for 14 d with one change of media and fresh addition of TNF alpha on day 8. The dishes were fixed with 10% formalin and stained with a 0.2% solution of crystal violet. To quantitate the amount of cells present in the dishes, the dishes were washed three times with PBS; the dye in the cells was eluted with 0.1 M sodium citrate (pH 4.2) and 50% ethanol for 30 min at room temperature and quantitated spectroscopically at 570 nm.

DNA was assayed as described by Labarca and Paigen using the fluorescent reagent, bisbenzimidazole (21). Protein was measured by the BCA protein assay reagent available from Pierce Chemical Co. (Rockford, IL) (22). These assays were determined in cells disrupted by sonication.

Effect of TNF alpha on differentiation of keratinocytes. The formation of cornified envelopes was quantitated using the method of King et al. (23). The cells in triplicate wells were exposed to 2 μCi of [^{35}S]-methionine/ml media for 48 h. The washed cells were then dissolved in 2 ml of 2% SDS, 20 mM DTT solution and the amount of radioactivity incorporated into the cross-linked, detergent-insoluble, cornified envelope was quantitated by liquid scintillation spectroscopy after collecting the envelopes on 10-μm pore size filters. These assays were performed in triplicate and each data point represented the mean of triplicate values from separate culture dishes.

Statistical analysis for the effects of TNF alpha were performed by analysis of variance using the appropriate programs in Systat (Systat, Inc., Evanston, IL).

Binding of TNF alpha to keratinocytes. Recombinant human TNF alpha, kindly supplied by Dr. Michael Shepard of Genentech, Inc., South San Francisco, CA, was iodinated by the iodogen method and purified as previously described (24). This method resulted in a fully biologically active form of ^{125}I-TNF alpha that remained stable for at least 4 wk. ^{125}I-TNF alpha had a specific activity of 800 Ci/mmol of protein.

To determine the extent of TNF alpha binding to keratinocytes, 400,000 cpm of ^{125}I-TNF alpha was incubated with confluent cultures of keratinocytes at 4°C, 24°C, and 34°C for up to 20 h. The nonspecific binding was determined by the ^{125}I-TNF alpha binding to keratinocytes in the presence of 100 nM nonlabeled TNF alpha. The medium was removed, the cells were rinsed three times with the binding buffer, and the cell-bound radioactivity was solubilized in 1% SDS and quantitated in a gamma counter. Nonspecific binding, determined in presence of excess (100 nM) TNF alpha, was subtracted from the total binding to determine the specific binding. To determine the degree to which the bound TNF alpha was internalized, the cells were incubated twice for 5 min each with 2 ml 0.05 M glycine-HCl, 0.15 M NaCl, pH 3.0, after the three rinses with binding buffer. The radioactivity in the combined acid washes was used to determine the surface-bound TNF alpha; the radioactivity in the subsequently solubilized (1% SDS) cells was used to determine the internalized TNF alpha.

For quantitating the receptor content and affinity, cells grown to confluence in 6-well multi-well plates were rinsed once with Eagle's MEM containing 10% FCS (binding buffer), and then incubated at 4°C with 400,000 cpm of ^{125}I-TNF alpha for 8 h in the presence of varying concentrations of unlabeled TNF alpha (25). The medium was removed, the cells were rinsed three times with the binding buffer, and the cell-bound radioactivity was solubilized in 1% SDS and quantitated in a gamma counter. Nonspecific binding, determined in the presence of excess (100 nM) unlabeled TNF alpha, was subtracted from total binding to calculate specific binding. Scatchard analysis was performed to determine the affinity and number of binding sites per cell. Total DNA was used to estimate the number of cells per well; 7.5 μg DNA was taken as equivalent to 1×10^6 cells since keratinocytes represent a normal human diploid cell line (26). Triplicate values for each data point were fit by computer to a single class of receptor sites using the linear regression program in Systat.

Results

Effect of TNF alpha on keratinocyte proliferation. To determine the effect of TNF alpha on keratinocyte growth, cells were exposed to 0.1–10.0 nM TNF alpha from the day after plating for 5, 8, or 22 d. The data are shown in Fig. 1. Growth inhibition was observed at TNF alpha concentrations > 1.0 nM. The longer the cells were exposed to TNF alpha the greater the inhibition of growth. 10 nM TNF alpha inhibited growth by as much as 50% after 8 d of exposure.

We then evaluated the effect of TNF alpha on keratinocytes at different stages of their growth. When TNF alpha (1.7 nM) was added to keratinocytes beginning at different times

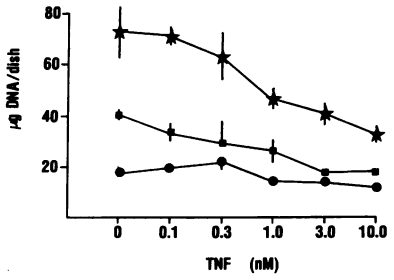

Figure 1. Effect of TNF alpha on keratinocyte proliferation. Keratinocytes were grown in the presence of various amounts of TNF alpha (0.1–10.0 nM) from 1 d after plating. Cells were harvested on day 5 (80% confluent) (●), day 8 (100% confluent) (■), or day 22 (2 wk postconfluent) (★). DNA was estimated in sonicated cell homogenates. Each point represents the mean±SD of triplicate determinations.

(0, 3, 5, 7, or 10 d) in culture, its effect on DNA content was found to vary according to the time it was added to the cells (Fig. 2). Addition of TNF alpha at the time of plating reduced plating efficiency by 13% (45 vs. 58% plating efficiency in TNF-treated and control cells, respectively). Addition of TNF alpha 24 h or more after plating had no effect on cell attachment as determined by the absence of cells in the medium at subsequent time points. Addition of TNF alpha to keratinocytes before confluence (day 6) inhibited the proliferation of these cells to a greater extent than addition of TNF alpha to cells after they had reached confluence. For example, cultures exposed to TNF alpha from day 3 or 5 had 37–45% reduction in DNA content at day 12 compared with controls, whereas cultures exposed to TNF alpha from day 7 or 10 had only a 3–20% reduction. Thus, the earlier and longer the cells are exposed to TNF alpha, the more profound the effect of TNF alpha on cell growth.

In clonal growth assays (Fig. 3), doses > 0.1 nM TNF alpha significantly reduced the number of colonies in a dose-dependent fashion. 0.3 nM TNF alpha reduced the number of cells by 50% and concentrations > 1.0 nM reduced the cell numbers by > 80%. The growth inhibitory effect of TNF alpha observed in clonal growth assays was more pronounced than the inhibitory effect of TNF alpha in reducing the DNA con-

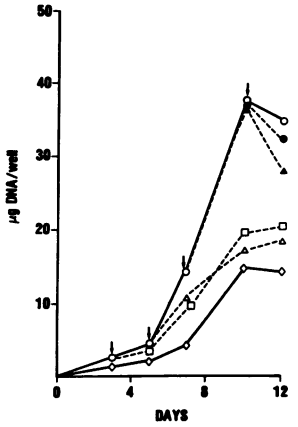

Figure 2. Effect of TNF alpha on keratinocyte proliferation at different stages of growth. 30 μg/ml TNF alpha (1.7 nM) was first added to the cells at different times as indicated by the arrows beginning on day 0 (◇), 3 (□), 5 (△), 7 (▲), or 10 (●), and the cells were grown for up to 12 d. Medium was changed and fresh TNF alpha added to the appropriate cultures on those same days. Control cultures (○) received no TNF. Throughout the 12-d incubation cells were harvested by scraping off into 1 ml PBS, and DNA was estimated in sonicated cell homogenates. For clarity of the figure, SD were omitted and each data point is expressed as the mean of triplicate determinations. All values for SD were either < 12% of mean or < 1.5 μg DNA. Multivariable analysis of variance indicated that TNF alpha had a significant ($P < 0.001$) inhibitory effect on DNA content which was significantly increased ($P = 0.002$) with time of incubation.

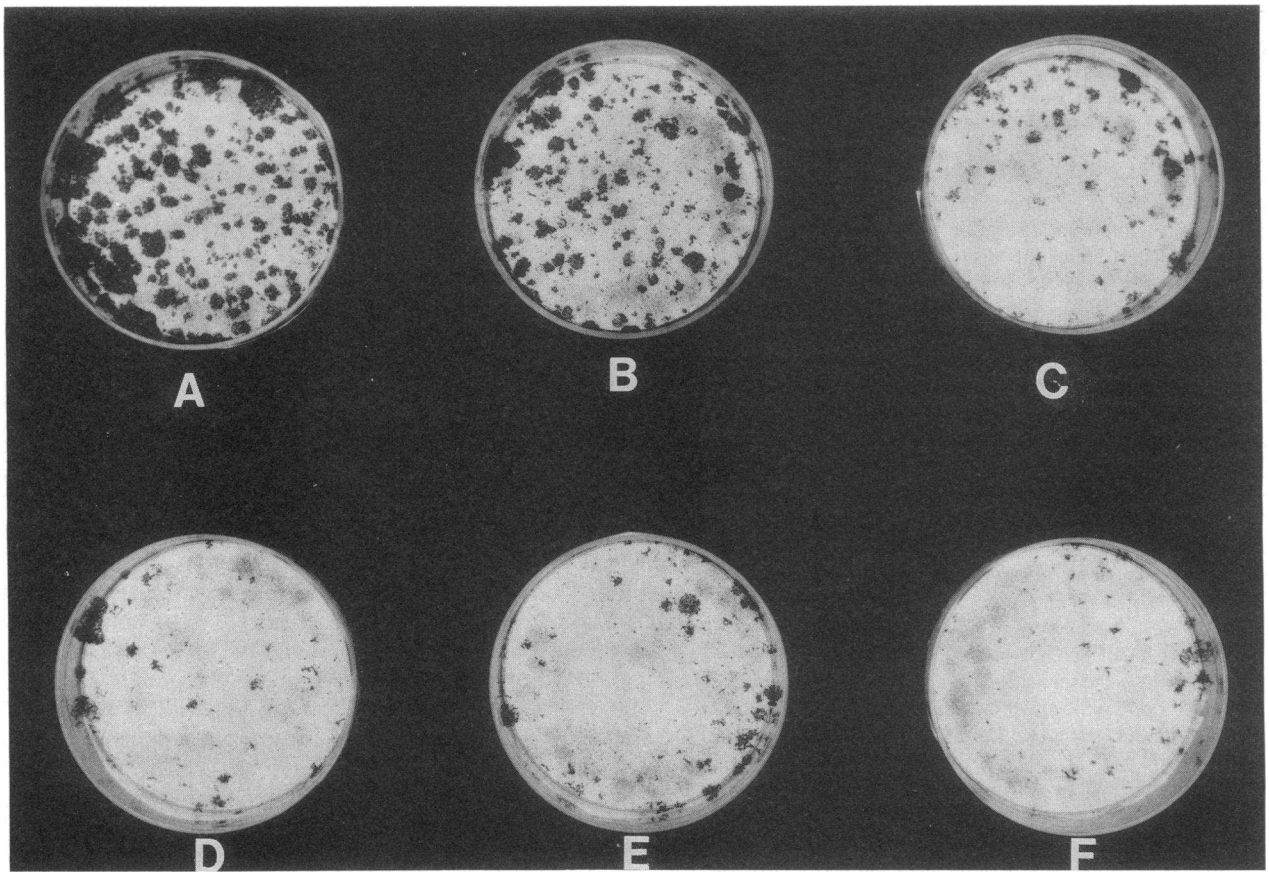

Figure 3. Effect of TNF alpha on clonal growth of keratinocytes. 10^4 keratinocytes were seeded into 60-mm dishes and grown in DME containing 5% FCS. 24 h after plating indicated amounts of TNF alpha were added to the dishes. The cells were grown for 14 d with one medium change on day 8 with fresh TNF alpha addition. On day 14 medium was changed and dishes were washed with PBS, fixed in 10% formalin, and stained for 15 min with 0.2% crystal violet. *A,* No TNF alpha; *B,* 0.1 nM TNF alpha; *C,* 0.3 nM TNF alpha; *D,* 1.0 nM TNF alpha; *E,* 3.0 nM TNF alpha; *F,* 10.0 nM TNF alpha.

tent as seen in Fig. 1. This may be due to the lower seeding density of the clonal assay compared with the other experiments (clonal assays had 1/10 number of cells plated). To determine whether these antiproliferative effects of TNF alpha on keratinocytes were reversible we carried out the experiment described in Fig. 4. Removal of TNF alpha from the medium after 5 d permitted the TNF alpha-exposed keratinocytes to achieve the same cell numbers as controls. The rate of growth was higher than controls initially after removal of TNF alpha, until equivalent cell densities were achieved. Thus, the inhibitory effects of TNF alpha on keratinocyte cell proliferation are reversible.

Effect of TNF alpha on keratinocyte differentiation. To assess whether the antiproliferative effects of TNF alpha were accompanied by a stimulation of differentiation, we measured the effect of TNF alpha on the cornified envelope formation, a marker of terminal differentiation of keratinocytes, as assessed by [^{35}S]methionine incorporation into the detergent-insoluble cornified envelopes. The cornified envelope formation was highest in confluent cultures (93 dpm/μg DNA in preconfluent cultures vs. 1,029 dpm/μg DNA in confluent cultures). Although postconfluent keratinocytes had a higher cornified envelope content, their rate of formation of cornified envelope was fivefold lower than that of confluent cells. The data in Fig. 5 are expressed as percent of control (cells grown in the absence of TNF alpha) to facilitate comparisons of the effect of TNF alpha on the cells at different stages of confluence. 0.3–3.0 nM TNF alpha stimulated the rate of cornified envelope formation more in the confluent than in the preconfluent keratinocytes but had no effect on postconfluent keratinocytes. Maximal stimulation at 1.0 nM TNF alpha was 219% in confluent cells and 155% in preconfluent cells.

Binding of TNF alpha to keratinocytes. To assess whether the biological effects of TNF alpha on keratinocytes were ac-

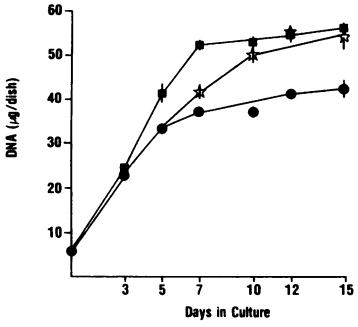

Figure 4. Reversibility of TNF alpha effect on keratinocyte growth. Keratinocytes were grown either in the absence (■) or presence of 1.0 nM TNF alpha (●) for 15 d. On day 5 some cells that received TNF alpha from day 1 were transferred to TNF alpha-free medium (★) and grown till day 15. Cells were harvested on different days as indicated, and DNA was assayed in triplicate.

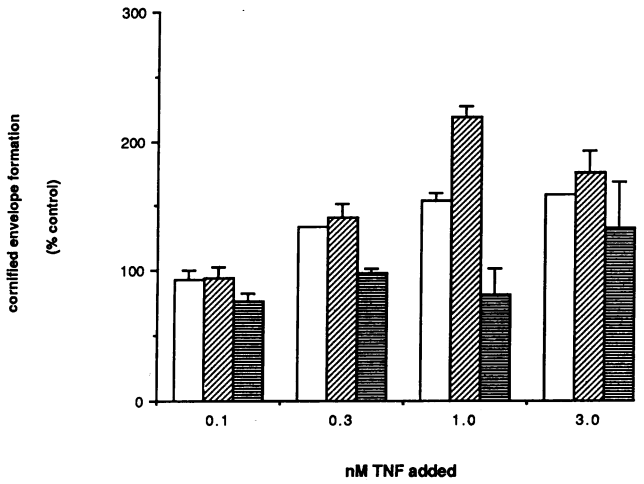

Figure 5. Effect of TNF alpha on differentiation of keratinocytes. 80% confluent (□), 100% confluent (▨), or 1 wk postconfluent keratinocytes (▤) were incubated for 48 h with 2 μCi/ml media [^{35}S]-methionine, and [^{35}S]methionine incorporation into cornified envelopes was measured as described in Methods. Data are expressed as % control (no TNF alpha)±SD. Incorporation of [^{35}S]methionine into cornified envelopes in preconfluent, confluent, and postconfluent cultures was 93±51, 1,029±139, and 201±21 dpm/μg DNA, respectively, in the control (no TNF alpha) dishes.

companied by its binding to keratinocytes, we determined the specific binding of TNF alpha to keratinocytes. Fig. 6 shows a time course of TNF alpha binding to confluent cultures of keratinocytes at 4°C. At this temperature the nonspecific binding was 5–8% of the total binding. Specific binding reached a plateau by 8 h. In other experiments (data not shown) maximal binding of TNF alpha was achieved by 2 h at 34°C and by 4 h at 24°C. However, 85–90% of the cell-associated radioactivity at 24 and 34°C was internalized, whereas the extent of internalization was only 20% at 4°C. Degradation of TNF alpha was also minimal at 4°C. Therefore, Scatchard analysis of TNF alpha binding to confluent cultures of keratinocytes was performed at 4°C for 8 h and is shown in Fig. 7. The Scatchard plot is shown in the inset of Fig. 7. Keratinocytes contained one class of high-affinity binding sites numbering 1,250/cell with a K_d of 0.28 nM.

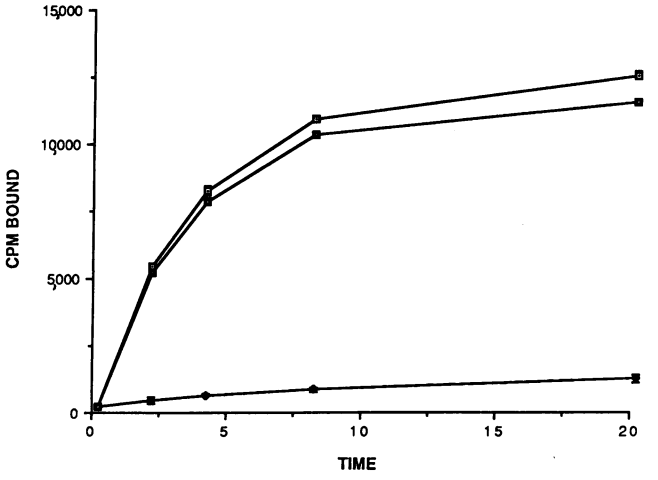

Figure 6. Binding of TNF alpha to confluent cultures of keratinocytes. Time course of binding at 4°C. 400,000 cpm ^{125}I-TNF alpha was incubated in a final vol of 1 ml DME containing 10% FCS for different times either in the absence (□) or in the presence of 100 nM nonlabeled TNF alpha (■). Cell-bound radioactivity was determined as described in Methods. Specific binding (▣) was determined by subtracting the nonspecific binding from the total binding. Data points are mean±SD of triplicate determinations for total and nonspecific binding.

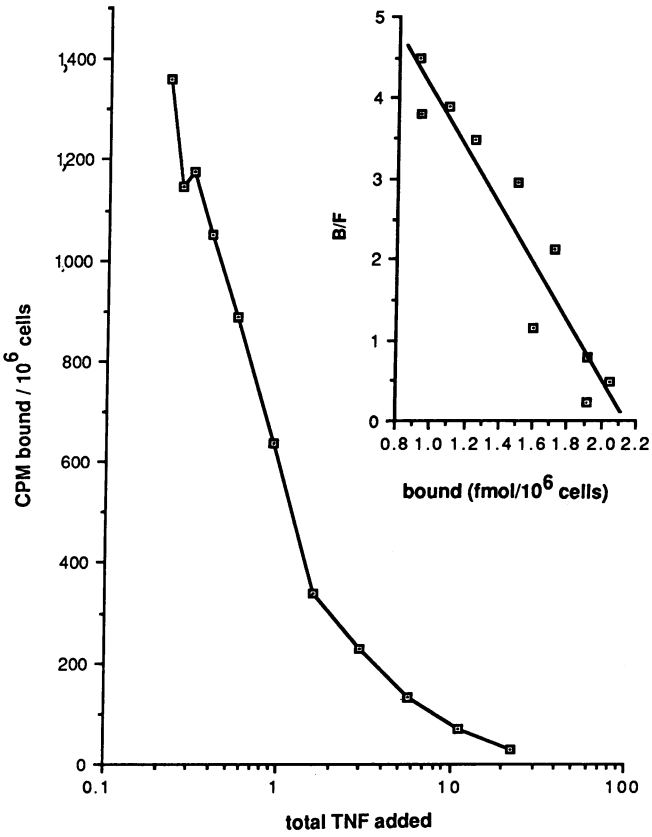

Figure 7. Effect of various concentrations of unlabeled TNF alpha on the binding of ^{125}I-TNF alpha to confluent cultures of keratinocytes. Cells were incubated with 400,000 cpm ^{125}I-TNF alpha for 8 h at 4°C in the presence of various concentrations of nonlabeled TNF alpha. The data were plotted as specific binding as a function of total amount of TNF alpha added (labeled + unlabeled). Scatchard analysis (*inset*) was done by calculating bound (fmol/10^6 cells) and bound/free over a range of TNF from 0.2 (tracer only) to 20 nM.

Discussion

In this study we investigated the effect of TNF alpha on normal human keratinocyte growth and differentiation and examined TNF alpha binding to these cells as a possible first step in mediating the biologic response. We have shown that TNF alpha at a concentration > 0.1 nM significantly inhibited proliferation of keratinocytes. These effects were reversible when TNF alpha was removed from the medium. The antiproliferative effects were most marked in the preconfluent stages of cell growth and were increased with time of exposure to TNF alpha. Accompanying this antiproliferative effect was a stimulation by TNF alpha of differentiation of the keratinocytes as indicated by the increased rate of formation of cornified enve-

lopes. The concentration of TNF alpha required to exert these biologic effects is comparable to the K_d of the TNF alpha receptor measured at 4°C (0.28 nM). Confluent cultures of keratinocytes rapidly internalized TNF alpha at 34 and 24°C. 90% of the cell-associated radioactivity at these temperatures was accounted for by the TNF alpha inside the cell by 2 h. This high rate of TNF alpha internalization is consistant with the in vivo observation (19) that the epidermis can accumulate a large proportion of parenterally administered TNF alpha.

TNF alpha regulates the growth and function of a wide variety of cells. In many tumor cell lines TNF alpha induces cell lysis (13, 27) although TNF alpha may actually promote growth in other cell lines (13). Metabolic effects of TNF alpha in nontransformed cell lines include inhibition of lipoprotein lipase activity in 3T3-L1 adipocytes and C2 myotubules (14), inhibition of lipid uptake and synthesis and stimulation of FFA release in 3T3-LI adipocytes (18), regulation of immune functions including induction of class I MHC antigens (15), induction of tissue procoagulant activity in endothelial cells (16), and induction of bone resorption and osteoclast formation in bone cell cultures (17). The number of TNF alpha receptors reported for these cells varies from 200 to 9,000, with affinities ranging from 1 to 30×10^{-10} M (13, 14, 27–33). In general the affinity of the TNF alpha receptor is appropriate for the potency of the biologic response in these cells, although not all cells with TNF alpha receptors have a detectable biologic response to TNF alpha (at least in terms of cell lysis) (13). As reported in our study, the keratinocyte receptor for TNF alpha is similar in affinity and number to other cell lines, but the response of the keratinocyte to TNF alpha in terms of differentiation (i.e., cornified envelope formation) is unique.

TNF alpha, in conjunction with other cytokines produced by fibroblasts and keratinocytes such as IL-1, may play a regulatory role in dermal-epidermal interactions during wound healing, inflammation, and epidermal growth and differentiation. It is not known whether TNF alpha is synthesized in keratinocytes, but it is conceivable that during inflammation or injury TNF alpha along with other cytokines such as IL-1 and IFN gamma are released into the circulation and accumulate in the skin. IFN, epidermal growth factor, and transforming growth factor beta are all known to effect proliferation and differentiation of keratinocytes (4–6); receptors for these cytokines have been demonstrated in keratinocytes (34–36). The observations that TNF alpha regulates keratinocyte growth and differentiation and that TNF alpha accumulates in the skin after in vivo administration suggest that TNF alpha has a physiologic role in intact epidermis.

Acknowledgments

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References

1. Green, H. 1977. Terminal differentiation of human epidermal cells. *Cell.* 11:405–416.

2. Watt, F. M. 1984. Selective migration of terminally differentiating cells from basal layer of cultured human epidermis. *J. Cell Biol.* 98:16–21.

3. Hennings, H., and K. A. Holbrook. 1983. Calcium regulation of cell-cell contact and differentiation of epidermal cells in culture: an ultrastructural study. *Exp. Cell Res.* 143:127–142.

4. Rheinwald, J. G., and H. Green. 1977. Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. *Nature (Lond.).* 265:421–424.

5. Bertolero, F., M. E. Kaigham, R. F. Camalier, and U. Saffiotti. 1986. Effect of serum and serum derived growth factors on growth and differentiation of mouse keratinocytes. *In Vitro Cell. Dev. Biol.* 22:423–428.

6. Yarr, M., R. L. Karassik, L. E. Schnipper, and B. A. Gilchrest. 1985. Effect of alpha and beta interferon on cultured human keratinocytes. *J. Invest. Dermatol.* 85:70–74.

7. Fuchs, E., and H. Green. 1982. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. *Cell.* 25:617–625.

8. Hosomi, J., J. Hosoi, E. Abe, T. Suda, and T. Kuroki. 1983. Regulation of terminal differentiation of cultured mouse epidermal cells by $1,25(OH)_2D$. *Endocrinology.* 113:1950–1957.

9. Smith, E. L., N. C. Walworth, and M. F. Holick. 1986. Effect of $1,25(OH)_2D$ on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum free condition. *J. Invest. Dermatol.* 86:709–714.

10. Hennings, H., K. Holbrook, and S. Yuspa. 1983. Factors influencing calcium induced terminal differentiation in cultured mouse epidermal cells. *J. Cell Physiol.* 116:265–281.

11. Hennings, H., D. Michael, C. Cheng, P. Steinert, K. Holbrook, and S. H. Yuspa. 1980. Calcium regulation of growth and differentiation of mouse epidermal cells. *Cell.* 19:245–254.

12. Voorhees, J. J. 1982. Cyclic AMP regulation of normal and psoriatic skin. *Arch. Dermatol.* 118:869–874.

13. Sugarman, B., B. B. Aggarwal, B. E. Hass, I. S. Figari, M. A. Palladino, and M. S. Shepard. 1985. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. *Science (Wash. DC).* 230:943–947.

14. Beutler, B., J. Mahoney, N. Le Trang, P. Pekala, and A. Cerami. 1985. Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *J. Exp. Med.* 161:984–995.

15. Chang, R. J., and S. H. Lee. 1986. Effects of interferon gamma and tumor necrosis factor-alpha on the expression of an Ia antigen on a murine macrolphage cell line. *J. Immunol.* 137:2853–2856.

16. Bevilacqua, M. P., J. S. Pober, G. R. Majeau, W. Fiers, R. S. Cotran, and M. A. Grimbrone, Jr. 1986. Recombinant TNF induce procoagulant activity in cultured human vascular endothelium: characterization and comparison with the action of interleukin-1. *Proc. Natl. Acad. Sci. USA.* 83:4533–4537.

17. Bertolini, D. R., G. E. Nedwin, T. S. Bringman, and G. R. Mundy. 1986. Stimulation of bone resorption and induction of bone formation in vitro by human tumor necrosis factors. *Nature (Lond.).* 319:516–518.

18. Patton, J. S., H. M. Shepard, H. Wilking, G. Lewis, B. B. Aggarwal, T. E. Eessalu, L. A. Gavin, and C. Grunfeld. 1986. Interferons and tumor necrosis factors have similar catabolic effects on 3T3-1 cells. *Proc. Natl. Acad. Sci. USA.* 83:8313–8317.

19. Beutler, B. A., I. W. Milsark, and A. Cerami. 1985. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. *J. Immunol.* 135:3972–3977.

20. Rheinwald, J. G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell.* 6:331–343.

21. Labarca, C., and K. Paigen. 1980. A simple, rapid and sensitive DNA assay procedure. *Anal. Biochem.* 102:344–352.

22. Publications number 23225. 1985. BCS protein assay reagent. Pierce Chemical Company, Rockford, IL.

23. King, I., S. L. Mella, and A. C. Sartorelli. 1986. A sensitive method to quantify the terminal differentiation of cultured epidermal cells. *Exp. Cell Res.* 167:252–256.

24. Aggarwal, B. B., T. E. Eessalu, and P. E. Hass. 1985. Character-

ization of receptors for human tumor necrosis factor and their regulation by gamma-interferon. *Nature (Lond.)*. 318:665–667.

25. Aggarwal, B. B., P. R. Traquina, and T. E. Eessalu. 1986. Modulation of receptors and cytotoxic response of TNF-alpha by various lectins. *J. Biol. Chem.* 261:13652–13656.

26. Patterson, M. K., Jr. 1979. Measurement of growth and viability of cells in culture. *Methods Enzymol.* LVIII:141–152.

27. Jadus, M. R., G. Schmmunk, J. Y. Djeu, and R. Parkman. 1986. Morphology and lytic mechanisms of interlukin 3-dependent natural cytotoxic cells: tumor necrosis factor as a possible mediator. *J. Immunol.* 137:2774–2783.

28. Wang, A. M., A. A. Creasey, M. B. Landner, L. S. Len, J. Strickler, V. N. Van Arsdell, R. Yamamoto, and D. F. Mark. 1985. Molecular cloning of complementary DNA from human TNF. *Science (Wash. DC)*. 228:149–150.

29. Nawroth, P. P., I. Bank, D. Handley, J. Cassimeris, L. Chess, and D. Stern. 1986. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin-1. *J. Exp. Med.* 163:1363–1375.

30. Gamble, J. R., J. M. Harlan, S. J. Klebanoff, and M. A. Vadas. 1985. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc. Natl. Acad. Sci. USA*. 82:8667–8671.

31. Tsujimoto, M., Y. K. Yip, and J. Vilcek. 1985. Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. *Proc. Natl. Acad. Sci. USA*. 82:7626–7630.

32. Scheurich, P., U. Ucer, M. Krouke, and K. Pfizenmair. 1986. Quantification and characterization of high affinity membrane receptor for TNF on human leukemic cell lines. *Int. J. Cancer*. 38:127–133.

33. Ruggiero, U., J. Tavernier, W. Fiers, and C. Baglioni. 1986. Induction of the synthesis of tumor necrosis factor receptors by interferon-gamma. *J. Immunol.* 136:2445–2450.

34. Carpenter, G., and J. G. Zendegui. 1986. Epidermal growth factor, its receptor, and related proteins. *Exp. Cell Res.* 164:1–10.

35. Nickloff, B. J. 1987. Binding of ^{125}I-gamma interferon to cultured human keratinocytes. *J. Invest. Dermatol.* 89:132–135.

36. Shipley, G. D., M. R. Pitelkow, J. J. Wille, R. E. Scott, and H. L. Moses. 1986. Reversible inhibition of normal human prokeratinocyte proliferation by type B transforming growth factor-growth inhibitor in serum-free medium. *Cancer Res.* 46:2068–2071.