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Epidermal Growth Factor and Transforming Growth Factor-Alpha Decrease Gamma Interferon Receptors and Induction of Intercellular Adhesion Molecule (ICAM-1) on Cultured Keratinocytes

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The link between the epidermal keratinocytes of the skin and the activated T lymphocytes of the immune system is mediated by a variety of cytokines, including gamma interferon (IFN-γ). We studied the influence of keratinocyte mitogens such as transforming growth factor-alpha (TGF-α), epidermal growth factor (EGF), and somatomedin-C (SM-C) on the ligand binding of ^{32}P-labelled IFN-γ to cultured keratinocytes derived from normal appearing adult human skin. Keratinocytes placed in a medium devoid of mitogens become growth arrested, and these quiescent cells expressed 2.4 times (28,900 versus 12,200 sites/cell) as many high affinity IFN-γ receptors (Kd = 0.22 nM) compared to keratinocytes which were actively growing in medium containing TGF-α (25 ng/ml) or EGF (10 ng/ml). The reduction in IFN-γ receptor sites by TGF-α/EGF was mitogen specific, as adding SM-C (500 ng/ml) did not have any effect on ligand binding, although it similarly stimulated keratinocyte growth. The reduction in IFN-γ receptors was time dependent, occurring primarily after 24–48 hours of change in tissue culture conditions. The reduction in the number of high affinity IFN-γ receptors by TGF-α/EGF had immunobiological consequences, because quiescent keratinocytes in basal medium had an increased expression of HLA-DR and intercellular adhesion molecule-1 (ICAM-1) induced by IFN-γ, compared to actively growing TGF-α/EGF treated keratinocytes. These results suggest that rapidly proliferating keratinocytes exposed to TGF-α/EGF but not SM-C are capable of altering their response to IFN-γ by decreasing their number of cell surface high affinity receptors for IFN-γ.

The interferons are a large family of glycoproteins which were initially identified because of their antiviral activity, but also have been found to be capable of modulating cellular proliferation and immunological reactions (reviewed in Lengyel, 1982). Within the last few years, the biochemistry and physiology of interferons has become of increasing interest among investigative dermatologists (Nickoloff, 1991b). The human receptor for gamma interferon (IFN-γ) has been cloned and expressed by several groups, which have discovered that there must exist an additional species-specific component, besides the receptor itself, necessary for functional activity (Aguet et al., 1988; Gray et al., 1989). Despite these elegant molecular biological investigations, relatively little is known regarding the modulation of IFN-γ receptor expression on the cell surface of keratinocytes, or other cell types (Nickoloff, 1987). Our laboratory for the past several years has been interested in elucidating the potentially important role of IFN-γ, and its receptor, on keratinocytes with particular emphasis on psoriasis (reviewed in Nickoloff, 1988). We have recently proposed that one abnormality in the cytokine network by which the hyperkinetic psoriatic keratinocytes may arise is via functional inactivation of the growth inhibitory pathway mediated by high affinity IFN-γ receptors on lesional keratinocytes (Nickoloff, 1991a).

Using normal cultured human keratinocytes, IFN-γ has been documented to reduce the number of high affinity EGF receptor sites by approximately 50% (Nickoloff and Mitra, 1989). In this study, we posed the reciprocal question: could epidermal growth factor (EGF) and/or TGF-α influence IFN-γ cell surface receptors on cultured keratinocytes?

MATERIALS AND METHODS

Keratinocyte culture

Primary keratinocyte cultures were obtained after informed consent from normal appearing skin of

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healthy adult volunteers without any skin disease, and ingesting no medication, using a Castroviejo keratome, and preparing a single cell suspension as previously described (Nickoloff et al., 1989). The freshly isolated epidermal cells were seeded into 35 mm plastic petri dishes (Lux, Flow Laboratories, Naperville, IL) and grown in the presence of a low calcium (0.15 mM), serum free medium containing EGF (10 ng/ml), insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), bovine pituitary extract 0.4% v/v, designated KGM (Clonetics Corp, San Diego, CA). Keratinocytes were passaged routinely with 0.03% trypsin/0.01% EDTA, and used between passage number 2 and 4.

Growth modulatory treatments

Rapidly growing undifferentiated keratinocytes maintained in KGM can be growth arrested at low cell densities by replacing the KGM with a basal medium devoid of the mitogens EGF, insulin, and pituitary extract (designated KBM; Clonetics Corp). Based on our previous work (Nickoloff, 1988), these quiescent cultures can be reactivated to proliferate by supplementing the KBM with either EGF (10 ng/ml; receptor grade, Collaborative Research, Inc., Bedford, MA), TGF-α (25 ng/ml; Collaborative Research, Inc.), or recombinant somatomedin-C (SM-C) which is also referred to as insulin-like growth factor-1 (500 ng/ml; Collaborative Research, Inc.). In a standard binding assay, keratinocytes at different seeding density (range = 4–6 × 10^4 cells) were placed in 24-well plates and grown in KGM to sub-confluency. Keratinocytes were washed 3 times with 1 ml KBM, and then placed in KBM for 18–24 hours. After this incubation with KBM, the keratinocytes were then exposed for the next 48 hours to either KBM alone, KGM, KBM + EGF (10 ng/ml), KBM + TGF-α (25 ng/ml), KBM + SM-C (500 ng/ml), or KBM + EGF (10 ng/ml) + SM-C (500 ng/ml).

Radiolabelling of IFN-γ

Radiolabelling of IFN-γ was accomplished using γ-^{32}P-ATP and the procedure described by Mariano et al. (1987) and Langer et al. (1986). Briefly, 1 μg of recombinant human IFN-γ (A.C. Feldman, Genentech, Inc., South San Francisco, CA) having a specific activity of 2.5 × 10^7 units/mg was incubated for 60 minutes at 30°C with 250 μCi of γ-^{32}P-ATP (Amersham Corp, Arlington Heights, IL; > 5,000 Ci/mmol), and 5 units of the catalytic subunit of cAMP-dependent protein kinase from bovine heart muscle (Sigma Chemical Co., St. Louis, MO; P-2645) in 30 μl of 20 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, 100 mM NaCl, and 12 mM $MgCl_2$. The reaction was terminated by cooling on ice and adding 600 μl of 5 mg/ml bovine serum albumin (Sigma Chemical Co.) in 10 mM sodium pyrophosphate (pH 6.7). Then 10 mM sodium pyrophosphate, pH 6.7 (3 liters), was used to dialyze the ^{32}P-labelled IFN-γ (^{32}P-IFN-γ) overnight at 4°C (dialysis tubing 12,000 molecular weight cutoff; Bethesda Research Labs, MD; #5961). After dialysis, the ^{32}P-IFN-γ was aliquoted into 100 μl fractions and stored at −80°C, and used within 2 weeks of labelling. The specific activity of the ^{32}P-IFN-γ after dialysis resulted in a representative yield of 980 Ci/mmol. The biological activity of the ^{32}P-IFN-γ was verified by comparing the ability of 1 U/ml, 5 U/ml, 10 U/ml, and 50 U/ml of ^{32}P-IFN-γ versus non-labelled IFN-γ to induce HLA-DR and ICAM-1 on cultured keratinocytes grown in 8-well Lab-Tek chambers. After 48 hr, the cells were fixed in acetone and immunostained for HLA-DR (Becton-Dickenson, Mountain View, CA) and ICAM-1 (RR1/1; Dr. T. Springer, Boston, MA) using a sensitive avidin-biotin detection system (Vectastain Kit, Vector-Labs, Burlingame, CA). After dialysis, ^{32}P-IFN-γ was also run on a 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with and without addition of 2-mercaptoethanol (5%) as previously described with appropriate molecular weight standards (Rashidbaigi et al., 1985). Following electrophoresis, the gel was dried and autoradiographed (Kodak X-Omat film).

Ligand binding assay

After the 48 hr growth and differentiation modulatory treatment, keratinocytes were washed 3 times over a 3 hr time interval in ligand binding buffer (Earle's balanced salt solution (EBSS), containing 0.2% bovine serum albumin, RIA grade, Sigma Chemical Co.). The cells were chilled on ice and incubated with 0.4 ml of binding buffer and varied concentrations of ^{32}P-IFN-γ and non-labelled IFN-γ. The cultures were maintained at 4°C for the duration of the binding assay (4 hr), which was terminated by washing the monolayers 3 times with 1.5 ml ice-cold binding buffer. Solubilization of the cells was accomplished using 1 ml of 0.1 N sodium hydroxide plus 1% sodium dodecyl sulfate (SDS), followed by liquid scintillation counting. Non-specific binding was determined by adding a thousand-fold excess of unlabelled IFN-γ to parallel sample wells, and was no greater than 25% of the total amount bound under any treatment conditions. Data points represent the average of triplicate samples with non-specific binding subtracted. Each figure represents the mean of three separate experiments. For each experimental condition, the number of keratinocytes per well was determined and kept to within 10% of the other wells by adjusting the seeding density, since keratinocytes maintained for 48 hr in KBM alone will not significantly increase in cell number, compared to those maintained in KGM or KBM plus EGF/TGF-α and/or SM-C. Maintaining the same relative cell density is critical, because the expression of cell surface receptors is influenced by the degree of confluency (Rizzino et al., 1988). The two-tailed Student's t-test was performed for statistical analysis of differences in receptor sites/cell.

Immunobiological consequences of reduced IFN-γ binding

To determine the possible immunobiological consequences of the growth modulatory treatments, keratinocytes maintained in either KGM, KBM, KBM + EGF, KBM + SM-C, or KBM + EGF +SM-C for 48 hr were exposed to IFN-γ (1 U/ml, 10 U/ml) for an additional 24 hr. These cells were then analyzed for cell surface expression of ICAM-1 and HLA-DR by preparing single cell suspensions (using 0.03% trypsin, 0.01% EDTA) and indirect immunofluorescence staining followed by FACS analysis as previously described (Nickoloff et al., 1989). Isotype control (Leu 2a, IgGl;

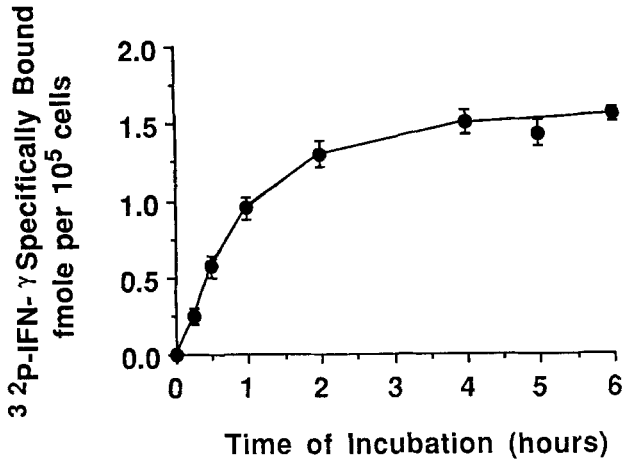

Fig. 1. Kinetics of specific ^{32}P-IFN-γ ligand binding to cultured keratinocytes maintained in KGM.

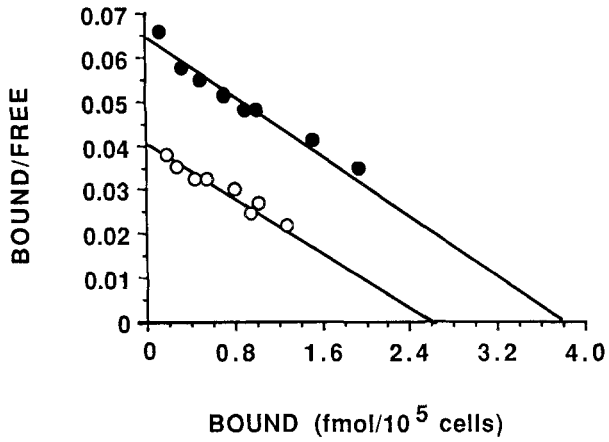

Fig. 2. Scatchard plot of ^{32}P-IFN-γ ligand binding to keratinocytes maintained in either KBM (solid circles) or KGM (open circles).

Becton-Dickenson, Mountain View, CA) was subtracted from the ICAM-1 and HLA-DR results.

RESULTS

Characterization of ^{32}P-IFN-γ

To establish that the procedure used to radiolabel the IFN-γ did not alter the IFN-γ, 2 studies were performed. First, the radiolabelled material was subjected to SDS-PAGE under reducing conditions which revealed that ^{32}P-IFN-γ migrates previously as a single band with an apparent molecular weight of approximately 17,000 daltons with a faint second band at 34,000 daltons (data not shown), in good agreement with previous investigators who have utilized the same labelling procedure (Rashidbaigi et al., 1985; Sarkar and Gupta, 1984). Secondly, the biological activity retained after phosphorylation was determined by adding equivalent concentrations of labelled and unlabelled IFN-γ (1, 5, 10, 50 U/ml) to keratinocytes grown in Lab-Tek chambers, and after 48 hr, examining the immunoperoxidase stained monolayers for ICAM-1 and HLA-DR expression by light microscopy. Both IFN-γ preparations produced equivalent results in the extent and intensity of keratinocyte ICAM-1/HLA-DR induction with a concentration dependent increase in the number of cells positively stained and in the intensity of staining (data not shown).

Time course of ^{32}P-IFN-γ binding

^{32}P-IFN-γ bound at 4°C specifically to keratinocytes grown in KGM with the binding reaching a plateau between 4 and 6 hr (Fig. 1), in good agreement with our previous studies using IFN-γ which had been labelled with ^{125}I using the Bolton-Hunter reagent (Nickoloff, 1987). Non-specific binding was determined by adding excess (thousandfold) non-radiolabelled IFN-γ, and was found to average between 15% and 25% of total binding at 4°C.

Scatchard analysis of binding

The specific binding of ^{32}P-IFN-γ to keratinocytes in KGM or KBM increased with increasing concentration of ^{32}P-IFN-γ and achieved saturation under both conditions between 200 and 400 pM (data now shown). Scatchard analysis of the ligand binding data yielded linear plots (regression coefficient = 0.9) indicating the presence of a single class of high affinity binding sites (Fig. 2). The dissociation constant for keratinocytes grown in KGM was calculated to be 0.25 ± .08 nM with 19,830 ± 880 receptor sites/keratinocyte.

The Scatchard plots of ligand binding for keratinocytes switched from KGM to other culture conditions revealed significant changes in the number of high affinity IFN-γ receptors, without significant alteration of the dissociation constants. Keratinocytes placed in KBM increased the number of sites/cell to 28,900 ± 1,136 ($P < .01$ compared to KGM) with a Kd = 0.22 ± .04 nM. Conversely, adding EGF (10 ng/ml) to the KBM reduced the number of sites/cell to 12,200 ± 820, and adding TGF-α (25 ng/ml) to KBM also reduced the number of high affinity sites/cell to 13,800 ± 1,261 ($P < .01$ for both results compared to KGM). This reduction was mitogen specific because adding SM-C (500 ng/ml) to KBM, which induces the approximately equivalent stimulation of keratinocyte growth as EGF (Turbitt et al., 1990), had no effect of lowering binding sites (25,800 ± 984). When EGF was combined with SM-C, the number of binding sites per cell was also reduced to 15,300 ± 964.

Kinetics of increased/decreased ^{32}P-IFN-γ binding by growth modulatory treatment

Because of our previous experience in which ^{125}I-EGF binding to cultured keratinocytes was inhibited by IFN-γ after 24 and 48 hr exposure (Nickoloff and Mitra, 1989), we performed a similar kinetic analysis measuring ^{32}P-IFN-γ binding. Figure 3 reveals that during the first 15 hr of incubation in either KGM, KBM, or KBM plus EGF there is no significant alteration in ^{32}P-IFN-γ binding. However, between 24 and 48 hr of incubation, the KBM had increased ^{32}P-IFN-γ binding compared to KGM, whereas the KBM plus EGF had decreased ^{32}P-IFN-γ binding. Thus, the kinetics of alteration in ^{32}P-IFN-γ binding are similar to changes in ^{125}I-EGF ligand binding. It should be emphasized

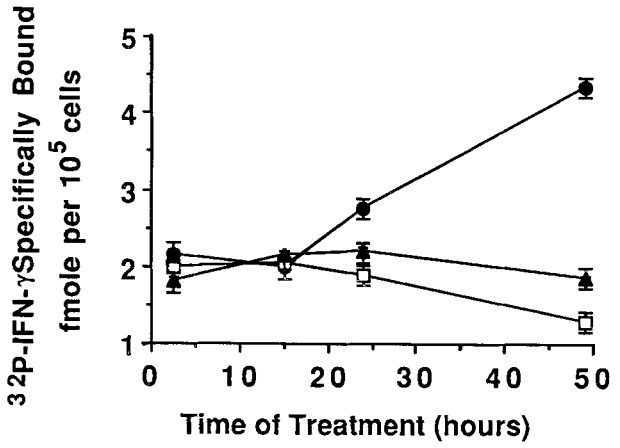

Fig. 3. Kinetics of change in ^{32}P-IFN-γ ligand binding for keratinocytes maintained in either KBM (solid circles), KGM (triangles), or KBM plus EGF (open squares).

TABLE 1. Induction of ICAM-1 and HLA-DR by IFN-γ after 24 hrs*

	IFN-γ = 1 U/ml		IFN-γ = 10 U/ml	
Culture conditions	ICAM-1	HLA-DR	ICAM-1	HLA-DR
KBM	39 ± 6	28 ± 5	112 ± 17	56 ± 8
KGM	18 ± 4	12 ± 4	68 ± 11	40 ± 6
KBM + EGF	21 ± 4	14 ± 5	57 ± 10	41 ± 7
KBM + SM-C	38 ± 6	30 ± 5	113 ± 14	59 ± 9
KBM + EGF + SM-C	15 ± 5	11 ± 5	67 ± 10	43 ± 7

*FACS analysis of keratinocyte cell surface expression of ICAM-1 and HLA-DR induced by IFN-γ under different growth conditions. Background, non-specific staining has been subtracted. Values represent mean channel flourescence readings (N = 4). EGF = 10 ng/ml; SM-C = 500 ng/ml.

that the seeding densities were adjusted appropriately, so that similar cell numbers per well (within 10%) were present at each time point studied for each different culture condition.

Immunobiological effects of growth modulatory treatments

To determine if the alterations in ^{32}P-IFN-γ binding had any immunobiological consequences, keratinocytes which had been previously maintained with the various growth modulatory treatments for 48 hr, were then exposed to IFN-γ (1 U/ml and 10 U/ml) for an additional 24 hr followed by FACS analysis for ICAM-1 and HLA-DR. As previously observed for keratinocytes maintained in KGM, a 24 hr exposure to IFN-γ results in greater ICAM-1 expression compared to HLA-DR (Griffiths et al., 1989b). Furthermore, keratinocytes maintained under conditions which were associated with reduced ^{32}P-IFN-γ ligand binding (i.e., KGM, KBM plus EGF, or KBM plus TGF-α) had decreased levels of ICAM-1 and HLA-DR induction, compared to keratinocytes with greater ^{32}P-IFN-γ binding (i.e., KBM) that had higher ICAM-1/HLA-DR expression (Table 1).

DISCUSSION

Interferons exert their pleiotropic biological effects by first binding to specific cell surface receptors (Langer and Pestka, 1988). Using 17,000 dalton, biologically active ^{32}P-labelled IFN-γ, a single class of high affinity IFN-γ receptors was demonstrated by Scatchard analysis to be present on multi-passaged human keratinocytes. The ligand binding studies performed at 4°C revealed a specific, time dependent (plateau at 4–6 hours), and saturatable reaction, with the resultant Kd and receptors/cell (0.25 nM; 19.8 × 10^3, respectively) to be within the range of values previously reported characterizing IFN-γ receptors on cells such as keratinocytes, fibroblasts, WISH cells, and monocyte/macrophages (Nickoloff, 1987; Mariano et al., 1987; Anderson et al., 1982; Finbloom et al., 1985; Sarkar and Gupta, 1984; Celada et al., 1984; Orchansky et al., 1986). Moreover, the current results indicate that the number of high affinity IFN-γ receptor sites/cell, as detected by ligand binding studies, was dependent on the growth characteristics of the cultured keratinocytes. Even though the relative density of the keratinocytes was maintained relatively constant throughout the studies, keratinocytes which were induced to become quiescent (using a basal medium devoid of supplemental mitogens) expressed a greater number of IFN-γ receptors compared to rapidly growing, undifferentiated keratinocytes. When quiescent cells were re-activated by adding the mitogens EGF or TGF-α, a time dependent decrease in the number of IFN-γ receptors was observed after 24–48 hr. This reduction was mitogen specific as IGF-1 (somatomedin-C) addition did not produce this reduction after 48 hr exposure. The ability of both EGF and TGF-α to have similar effects on keratinocytes is not surprising since they share substantial amino acid sequence homology and bind to the same EGF surface receptor (Marquardt et al., 1984).

The alterations in the number of keratinocyte high affinity IFN-γ receptors had immunobiological significances, because the relative degree of induction of ICAM-1 and HLA-DR by IFN-γ following treatments which either increased or decreased receptor numbers revealed parallel changes. For example, keratinocytes made quiescent by being placed in KBM, which had a greater number of IFN-γ receptors as detected by ligand binding, also had an increased amount of ICAM-1/HLA-DR induced by IFN-γ, compared to rapidly growing cells maintained in KGM. Conversely, when EGF or TGF-α was added to the KBM to become reactivated with diminished IFN-γ ligand binding, there was decreased ICAM-1/HLA-DR induction by IFN-γ. These in vitro immunobiological alterations are similar to those previously reported for human thyroid epithelial cells (Todd et al., 1990) in which EGF or TGF-α (2–50 ng/ml) inhibited the ability of IFN-γ (1–2.5 U/ml) to induce HLA-DR expression. The ability of mitogens such as EGF to increase IFN-γ production by T lymphocytes (Abdullah et al., 1989), and at the same time reduce keratinocyte IFN-γ receptors, may be part of an important reciprocal regulatory system operative between immunocytes and epithelial cells.

The modulation of keratinocyte growth in vitro includes pro-proliferation induced by TGF-α or EGF, and anti-proliferation by IFN-γ (Nickoloff et al., 1988). Previously, we established that IFN-γ down modulated high affinity keratinocyte EGF receptors (Nickoloff and Mitra, 1989), and this current work demonstrates that EGF/TGF-α can down modulate high affinity

IFN-γ receptors. Taken together, these results suggest that there are highly reciprocal interactions between these grown modulatory systems operative in keratinocytes (Nickoloff, 1991a). These molecular interactions involving TGF-α/EGF receptors and IFN-γ/IFN-γ receptors on cultured keratinocytes may help explain several features of the psoriatic epidermis. IFN-γ is detectable in psoriatic epidermis (Barker et al., 1991), yet there is significant keratinocyte hyperplasia, and only focal HLA-DR and ICAM-1 expression (reviewed in Nickoloff, 1991a). This is surprising because keratinocytes in vitro are extraordinarily sensitive to IFN-γ, being substantially growth inhibited (Nickoloff et al., 1984), and induced to strongly and diffusely express HLA-DR/ICAM-1 (Griffiths et al., 1989a,b). It has also been demonstrated that psoriatic epidermis contains markedly elevated TGF-α (Turbitt et al., 1990; Nickoloff et al., 1991). Based on the current results, we can postulate this increased TGF-α may be responsible for down-modulating keratinocyte IFN-γ receptors, thus rendering the hyperkinetic cells less responsive to the antiproliferative and immunomodulatory effects of IFN-γ (Nickoloff et al., 1989; Baker et al., 1988). Future work aimed specifically at determining whether there are decreased numbers of high affinity cell surface IFN-γ receptors on keratinocytes within lesions of psoriasis seems indicated (Nickoloff, 1991a). Clearly, much additional work remains to be performed to more fully understand the complex, reciprocal, concentration dependent, and time dependent molecular interactions involving the aforementioned ligand/receptor combinations in cultured normal keratinocytes, and in diseases such as psoriasis.

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LITERATURE CITED

Abdullah, N.A., Torres, B.A., Basu, M., and Johnson, H.M. (1989) Differential effects of epidermal growth factor, transforming growth factor-α, and vaccinia virus growth factor in the positive regulation of IFN-γ production. J. Immunol., *143:*113–117.

Aguet, M., Dembic, Z., and Merlin, G. (1988) Molecular cloning and expression of the human interferon-γ receptor. Cell, *55:*273–280.

Anderson, P., Yip, Y.K., and Vilcek, J. (1982) Specific binding of ^{125}I-human interferon-gamma to high affinity receptors on human fibroblasts. J. Biol. Chem., *257:*11301–11303.

Baker, B.S., Powles, A.V., Valdimarsson, H., and Fry, L. (1988) An altered response by psoriatic keratinocytes to gamma interferon. Scand. J. Immunol., *28:*735–740.

Barker, J.N.W.N., Karabin, G.D., Stoof, T.J., Sarma, V.J., Dixit, V.M., and Nickoloff, B.J. (1991) Detection of interferon-gamma mRNA in psoriatic epidermis by polymerase chain reaction. J. Dermatol. Sci., *2:*106–111.

Celada, A., Gray, P.W., Rinderknecht, E., and Schreiber, R.D. (1984) Evidence for a gamma interferon receptor that regulates macrophage tumoricidal activity. J. Exp. Med., *160:*55–74.

Finbloom, D.S., Hoover, D.L., and Wahl, L.M. (1985) The characteristics of binding of human recombinant interferon-gamma to its receptor on human monocytes and human monocyte-like cell lines. J. Immunol., *135:*300–305.

Gray, P.W., Leong, S., Fennie, E.H., Farrar, M.A., Pingel, J.T., Fernandez-Luna, J., and Schreiber, R.D. (1989) Cloning and expression of the cDNA for the murine interferon γ receptor. Proc. Natl. Acad. Sci. U.S.A., *86:*8497–8501.

Griffiths, C.E.M., Voorhees, J.J., and Nickoloff, B.J. (1989a) Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: Modulation by recombinant gamma interferon and tumor necrosis factor. J. Am. Acad. Dermatol., *20:*617–629.

Griffiths, C.E.M., Voorhees, J.J., and Nickoloff, B.J. (1989b) Gamma interferon induces different keratinocyte cellular patterns of expression of HLA-DR, DQ and intercellular adhesion molecule-1 (ICAM-1) antigens. Br. J. Dermatol., *120:*1–8.

Langer, J.A., and Pestka, S. (1988) Interferon receptors. Immunol. Today, *9:*393–400.

Langer, J.A., Rashidbaigi, A., and Pestka, S. (1986) Preparation of ^{32}P-labeled murine immune interferon and its binding to the mouse interferon receptor. J. Biol. Chem., *261:*9801–9804.

Lengyel, P. (1982) Biochemistry of interferons and their actions. Annu. Rev. Biochem., *51:*251–279.

Mariano, T.M., Kozak, C.A., Langer, J.A., and Pestka, S. (1987) The mouse immune interferon receptor gene is located on chromosome 10. J. Biol. Chem., *262:*5812–5814.

Marquardt, H., Hunkapiller, M.W., Hood, L.E., and Todaro, G.J. (1984) Rat transforming growth factor type I: Structure and relation to epidermal growth factor. Science, *223:*1079–1082.

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

Nickoloff, B.J. (1988) The role of interferon-γ in cutaneous trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. Arch. Dermatol., *124:*1835–1843.

Nickoloff, B.J. (1991a) The cytokine network in psoriasis. Arch. Dermatol. *127:*871–884.

Nickoloff, B.J. (1991b) Interferon and cutaneous metabolism. In: Biochemistry and Physiology of the Skin. L. Goldsmith, ed. Oxford University Press, New York, (in press).

Nickoloff, B.J., Basham, T.Y., Merigan, T.C., and Morhenn, V.B. (1984) Antiproliferative effects of recombinant alpha and gamma interferons on cultured human keratinocytes. Lab. Invest., *51:*697–701.

Nickoloff, B.J., Karabin, G.D., Barker, J.N.W.N., Griffiths, C.E.M., Sarma, V., Mitra, R.S., Elder, J.T., Kunkel, S.L., and Dixit, V.M. (1991) Cellular localization of interleukin-8 and its inducer tumor necrosis factor-alpha in psoriasis. Am. J. Pathol., *138:*129–140.

Nickoloff, B.J., and Mitra, R.S. (1989) Inhibition of ^{125}I-epidermal growth factor binding to cultured keratinocytes by antiproliferative molecules gamma interferon, cyclosporin A, and transforming growth factor-beta. J. Invest. Dermatol., *93:*799–803.

Nickoloff, B.J., Mitra, R.S., Elder, J.T., Fisher, G.J., and Voorhees, J.J. (1989) Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factor-α production in keratinocytes cultured from psoriatic lesions. Br. J. Dermatol., *121:*161–174.

Nickoloff, B.J., Mitra, R.S., Riser, B.L., Dixit, V.M., and Varani, J. (1988) Modulation of keratinocyte motility. Correlation with production of extracellular matrix molecules in response to growth promoting and antiproliferative factors. Am. J. Pathol., *132:*543–551.

Orchansky, P., Rubinsten, M., and Fischer, D.G. (1986) Interferon-gamma receptor in human monocytes is different from the one in non-hematopoietic cells. J. Immunol., *136:*169–173.

Rashidbaigi, A., Kung, H., and Pestka, S. (1985) Characterization of receptors for immune interferon in U937 cells with ^{32}P-labeled human recombinant immune interferon. J. Biol. Chem., *260:*8514–8519.

Rizzino, A., Kazakoff, P., Ruff, E., Kuszynski, C., and Nebelsick, J. (1988) Regulatory effects of cell density on the binding of transforming growth factor β, epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor. Cancer Res., *48:*4266–4271.

Sarkar, F.H., and Gupta, S.L. (1984) Receptors for human gamma interferon-binding and crosslinking of ^{125}I-labeled recombinant human gamma interferon. Proc. Natl. Acad. Sci. U.S.A., *81:*5160–5164.

Todd, I., Hammon, L.J., James, R.F.L., Feldmann, M., and Bottazzo, G.F. (1990) Epidermal growth factor and transforming growth factor-alpha suppress HLA class II induction in human thyroid epithelial cells. Immunol., *69:*91–96.

Turbitt, M.L., Akhurst, R.J., White, S.I., and Mackie, R.M. (1990) Localization of elevated transforming growth factor-alpha in psoriatic epidermis. J. Invest. Dermatol., *95:*229–232.