British Journal of Dermatology (1989) **121**, 161–174.

Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factor-α production in keratinocytes cultured from psoriatic lesions

B.J.NICKOLOFF, R.S.MITRA, J.T.ELDER,* G.J.FISHER* AND J.J.VOORHEES*

Departments of Pathology and *Dermatology, University of Michigan Medical Center, Ann Arbor, Michigan, U.S.A.

Accepted for publication 7 February 1989

SUMMARY

Keratinocytes from involved psoriatic plaques (PP), uninvolved, clinically symptomless skin of psoriatic patients (PN) and normal healthy skin (NN) have been cultured in a low calcium serum free system for multiple passages. In this way, the keratinocytes were removed from micro-environmental factors present in the skin. While the basal rate of proliferation of the PP, PN and NN keratinocytes was not different, the PP cells produced more transforming growth factor-α (TGF-α) than NN cells, and the antiproliferative response of PP cells to gamma interferon (IFN-γ), a product of activated T lymphocytes, was reduced. We studied IFN-γ because it can inhibit the proliferation of NN keratinocytes, induce their differentiation and the appearance of two immunoregulatory cell surface molecules, HLA-DR and intercellular adherence molecule-1 (ICAM-1), and because in another epithelial cell system, epidermal growth factor (EGF) modulates IFN-γ activity. The mean antiproliferative effects of IFN-γ at 50, 200, and 500 U/ml for the PP group ($n = 10$) was less compared to the NN group ($n = 11$); $P < 0{\cdot}001$, while the PN group ($n = 5$) had a less dramatic, but statistically significant, reduction in growth inhibition by IFN-γ only at 200 and 500 U/ml compared to NN cells; $P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively. The amount of TGF-α produced and secreted by PP keratinocytes from five different individuals was significantly greater than by NN keratinocyte cultures. In addition, IFN-γ induced TGF-α to a lesser extent in PP keratinocytes compared to NN keratinocyte cultures. Keratinocytes isolated from atopic dermatitis and Sézary syndrome patients were similar to NN keratinocytes.

In contrast to its differential effects on TGF-α production and proliferation, IFN-γ induced similar amounts of HLA-DR and ICAM-1 on PP, PN and NN keratinocytes. Thus, for the PP

Correspondence: B.J.Nickoloff, Department of Pathology, M4232 Medical Science I, 1301 Catherine Road, Ann Arbor, MI 48109-0602, U.S.A.

Presented at the National Meeting of the Society of Investigative Dermatology, April 28, 1988. Washington, D.C.

keratinocytes, there was a dissociation between the antiproliferative and immunomodulatory effects of IFN-γ. These results support our previous hypothesis that the hyperproliferation and altered differentiation of keratinocytes in psoriatic plaques is linked to an altered responsiveness of the keratinocytes to IFN-γ. Moreover, these results provide an *in vitro* correlate of our *in vivo* observation of increased TGF-α levels in psoriatic plaques. A new pathophysiological model to understand psoriasis is proposed which integrates these observations involving IFN-γ and TGF-α. This experimental approach also provides a system to dissect biochemical pathways of pathophysiological importance for keratinocyte hyperproliferation in psoriasis.

The hyperproliferation of keratinocytes in the epidermis has been regarded as the most characteristic feature of psoriasis.[1,2] Whether the primary cellular alteration resides in the keratinocyte, or in other cells in the skin such as fibroblasts, inflammatory cells or neurovascular cells which may produce diffusible factors that stimulate the keratinocyte in psoriasis is unknown.[3] One approach has been to isolate the epidermis and examine the inherent proliferative capacity of keratinocytes *in vitro*, apart from their cellular neighbours comprising the cutaneous micro-environment. This has produced conflicting results. Two reports using outgrowth cultures demonstrated that psoriatic keratinocytes incorporated more ^{3}H-thymidine into DNA[4] and hyperproliferated[5] compared to normal skin cells. However, subsequent to the first report, it was suggested that [^{3}H]-thymidine incorporation by cultured keratinocytes may not accurately reflect keratinocyte proliferation.[6] Another report demonstrated that keratinocytes cultured from uninvolved psoriatic epidermis had increased DNA synthesis compared to normal keratinocytes as determined by the autoradiographic labelling index.[7] At least four other reports have failed to observe any difference in the rate of proliferation when comparing keratinocytes from psoriatic lesions to normal skin cells.[8–11]

Despite the lack of a reproducible difference in cell proliferation *in vitro* between keratinocytes obtained from a psoriatic plaque (PP), or uninvolved skin from a patient with psoriasis (PN) and normal skin of a non-psoriatic (NN), Liu and Parsons suggested that there may be differences in the response of these cells to agents that inhibit cell growth.[11] One such agent that we have previously suggested may play a role in psoriasis is gamma interferon (IFN-γ), which is a secreted product of activated T lymphocytes.[12] We have reported that IFN-γ is a potent inhibitor of cultured normal keratinocyte proliferation and is an inducer of HLA-DR expression and keratinocyte differentiation.[12–15] Since IFN-γ is known to be present in psoriatic lesions[16] and since IFN-γ may be functioning as a feedback growth inhibitor in other normal cell systems,[17] it is possible that the hyperproliferation, altered differentiation and lack of HLA-DR expression in psoriatic plaques reflects an altered (decreased) responsiveness of the keratinocytes to IFN-γ in psoriasis.[18] To directly test this hypothesis, we have grown keratinocytes from 6 mm punch biopsies of PP, PN and NN skin as well as foreskin keratinocytes in a low calcium, serum free system.

Studies involving epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) were performed because EGF and TGF-α have been observed with another epithelial cell system, the thyrocyte, to be capable of inhibiting the action of IFN-γ.[19] Furthermore, TGF-α has been found to be present[20] and over-expressed at the levels of mRNA and protein in psoriatic plaques,[21] and has been suggested to be of importance in psoriasis.[22]

METHODS

IFN-γ

IFN-γ was obtained from Dr M. Shepard (Genentech, Inc., S. San Francisco, CA, U.S.A.) and had a specific activity of $1{\cdot}7 \times 10^7$ U/mg. The activity of the IFN-γ preparation utilized was continuously monitored using a sensitive and specific radioimmunoassay (Centocor, Malvern, PA, U.S.A.).

Keratinocyte culture

Punch biopsies of skin, 6 mm thick, were obtained after local anaesthesia (1% lidocaine). The biopsy sites and relevant clinical information on the psoriasis patients is listed in Table 1. Biopsies from the erythematous peripheral portion of the plaque were obtained from 10 consecutive psoriatic patients and in five of these patients, a biopsy at least 10 cm away from any lesion was also obtained and considered to represent uninvolved (PN) skin. Nine healthy adults (aged 25–58 years) without any skin disease and not using any topical or systemic medication were also biopsied. A 6 mm punch biopsy of lesional skin from a patient with atopic dermatitis and a patient with leukemic phase of cutaneous T-cell lymphoma (CTCL), Sézary syndrome, were also used as control examples of benign and malignant inflammatory dermatoses to compare to PP and PN keratinocytes. All biopsies were obtained after informed consent and with the approval of the University of Michigan Human Subjects Committee. After removal from the patient, the 6 mm punch biopsies were immediately placed in holding medium consisting of Earle's balanced salt solution (Gibco, Grand Island, NY, U.S.A.) containing penicillin/streptomycin/mycostatin (Gibco). The 6mm punch biopsy was bisected and placed in 5 ml of trypsin (0·3%), EDTA (0·1%) for 2–3 h at room temperature. After this incubation period, the epidermis was vigorously scraped with a sterile scalpel blade into 15 ml of a low calcium (0·15 mM), serum free medium (KGM, containing 10 ng/ml EGF, 5 μg/ml insulin, 0·5 μg/ml hydrocortisone, and 0·4% v/v bovine pituitary extract; Clonetics Corp., San Diego, CA, U.S.A.). It was immediately centrifuged and the pellet resuspended in 5 ml of KGM with vigorous pipetting to produce a single cell suspension which was seeded onto a single 60 mm plastic Petri dish (Lux, Flow Labs, Naperville, IL, U.S.A.). Generally, after 2–3 days small

TABLE 1. Psoriasis patient profile*

Donor	Age	Sex	Age-onset	% Body involvement	Time interval since last RX
PP1	46	M	30	25	3 months
PP2	23	M	13	50	10 months
PP3	50	M	38	80	3 months
PP4	34	F	21	60	5 months
PP5, PN5	35	M	26	60	6 months
PP6, PN6	37	M	24	70	5 months
PP7, PN7	53	F	20	60	7 years
PP8, PN8	60	F	25	60	5 months
PP9, PN9	34	F	13	75	6 months
PP10	40	M	16	85	12 months

* All biopsies of plaques were taken from the lower back and thigh regions.

clusters of adherent cells were apparent and the KGM was changed every 2–3 days with the subsequent appearance of progressively larger colonies of keratinocytes.

Two neonatal foreskins were obtained within 1–3 h of circumcision and placed in holding medium as described above. Using a Castroviejo keratome, 0·1 mm thick slices of the skin were obtained and placed in trypsin (0·3%), EDTA (0·1%) for 15–30 min at 37°C, followed by scraping of the epidermis and processing as above.

Cell proliferation studies

When the primary cultures of PP, PN and NN keratinocytes in the 60 mm dishes were subconfluent (10–14 days), they were passaged into 10 cm plastic Petri dishes (Corning, Corning, NY, U.S.A.) using 0·03% trypsin, 0·01% EDTA (5 min, 37°C). The KGM (8 ml) was replaced every 2–3 days and these cells were considered to be passage No. 1. To begin a cell proliferation assay, when the keratinocytes in the 10 cm dish were subconfluent (approximately $1·5–2·0 \times 10^6$ cells/plate), they were removed as described above and seeded ($6–8 \times 10^4$ cells) into 35 mm dishes. In a typical experiment, 15 35-mm plates were seeded and, after allowing the cells to attach and spread overnight, the unattached cells (< 25%) and medium was aspirated and replaced with fresh KGM with or without IFN-γ. The day of adding IFN-γ was designated as day 0. Triplicate dishes containing either 0, 50, 200 and 500 U/ml of IFN-γ were maintained in a humidified incubator at 37°C for 4 days without further change of medium. It should be noted that the initial keratinocytes not seeded into the 35 mm Petri dishes were passaged and placed into 10 cm Petri dishes to maintain the culture for subsequent proliferation assays. On day 4, the medium was removed and the cells washed once with fresh KGM and the keratinocytes were detached using 0·03% trypsin, 0·01% EDTA (5 min at 37°C) and an aliquot was placed into a haemocytometer for manual counting using a phase contrast microscope as previously described.[14] The cell counts are expressed as the mean +/− SEM. At least six separate counts were made for each plate. At least three different experiments were performed on passage Nos. 2–8 for each type of keratinocyte (i.e. PP, PN and NN) from each donor. The NN keratinocytes (either foreskin or adult skin) were matched by passage number with the PP and PN keratinocytes and were always within 1–2 passages. The remaining keratinocytes were centrifuged and the pellet was prepared for indirect immunofluorescence staining as described below.

Indirect immunofluorescence staining/FACS analysis

The cell pellets were resuspended in PBS containing 5% FCS (Hyclone) and indirect immunofluorescence staining was performed as previously described.[13] Briefly, $0·5 \times 10^6$ keratinocytes were indirectly stained for 20 min on ice with 0·5 μg of anti-HLA-DR monoclonal antibody TS1·16 (Gift from Drs C. Clayberger and A. Krensky, Stanford University), anti-intercellular adherence molecule-1, (ICAM-1) (RR 1/1; Gift from Dr T. Springer, Dana Farber Cancer Institute) and OKT8 (Ortho Diagnostics, Rareville, NJ, U.S.A.) diluted in heat-inactivated FCS containing 0·1% sodium azide. All of these primary antibodies are of the IgG_1 isotype. Detection of ICAM-1 was performed because we have previously observed that IFN-γ induced ICAM-1 on NN keratinocytes.[23] The keratinocytes were then washed twice and stained for 20 min with 0·5 μg of fluorescein isothiocyanate-conjugated (FITC) goat anti-mouse IgG monoclonal antibody (Tago, Burlingame, CA, U.S.A.). The keratinocytes were then washed twice more and fixed with freshly prepared 1% paraformaldehyde in PBS. The fluorescence per cells was determined using a fluorescence activated cell sorter (EPICS 54;

Coulter Corp.) and a histogram showing the number of stained cells versus the intensity of fluorescence was recorded.

Measurement of TGF-α protein

To determine the amount of TGF-α produced by cultured keratinocytes, semiconfluent keratinocytes (approximately $5–6 \times 10^5$ cells/35 mm dish) containing 1·5 ml of KGM were maintained for 48 h at 37°C, and the conditioned medium was assayed for TGF-α using a radioimmunoassay kit (Biotope, Seattle, WA, U.S.A.). Briefly, duplicate 100 μl aliquots were removed and immediately reduced and denatured as per the manufacture's recommendation. The samples were run with at least five different known TGF-α standards. No cross reactivity of the assay system for EGF was noted up to 1 μg/ml.

To determine the amount of TGF-α that may have been bound to the keratinocyte cell surface, thoroughly washed keratinocytes were exposed to a 'cold acid wash' in 50 mM glycine, 100 mM NaCl; pH 3·0 for 4 min at 4°C as previously described.[24]

Measurement of TGF-α mRNA

Keratinocytes ($2–4 \times 10^6$ per 100 mm dish) were lysed with 4 M guanidinium isothiocyanate, 5 mM sodium citrate, pH 7·0, 100 mM β-mercaptoethanol, 0·5% sodium sarcosinate, and RNA was isolated by overnight centrifugation at 100 000 × ***g*** over 5·7 M CsCl in 100 mM EDTA, pH 7·0.[25] RNA concentration was determined by absorbance at 260 nm and confirmed by non-denaturing agarose gel electrophoresis as previously described.[26] RNAs were size-fractionated by electrophoresis on a 1% formaldehyde-agarose gel,[25] and transferred to derivatized nylon membrane (Zeta-probe, Bio-Rad) in 10 × SSC, (1 × SSC = 0·15 M sodium chloride, 15 mM sodium citrate) as recommended by the manufacturer. Filters were hybridized against ^{32}P-labelled probes prepared by random priming[27] exactly as previously described.[28] The TGF-α cDNA probe psp65C17N3 was generously provided by Dr R. Derynck of Genentech, Inc.[22] The chicken glyceraldehyde-3-phosphate dehydrogenase (GAD) cDNA probe was derived from pGAD 28 and served to control for RNA loading and intactness.[29] Specific hybridization was estimated by subjecting the autoradiographs of the blots to laser scanning densitometry as previously described.[28]

Statistical analysis

The two-tailed Student's *t*-test for unpaired samples was performed.

RESULTS

Antiproliferative effect of IFN-γ on NN, PN and PP keratinocytes

On day 0, there were approximately $5–6 \times 10^4$ keratinocytes per 35 mm dish which increased to approximately $4–7 \times 10^5$ cells/dish by day 4 (approximately three population doublings). While there was some variation ($< 20\%$) in the exact degree of proliferation between days 0 and 4 for all the different types of keratinocytes (i.e. PP, PN, NN-adult and foreskin), approximately three population doublings were consistently observed throughout multiple passages of the different keratinocytes, although there was a tendency for proliferation to decline in later passages (data not shown). Since there was some variation in the degree of proliferation of the different types of keratinocytes in the absence of IFN-γ, we have expressed the data for the cultures treated with IFN-γ as a relative percentage of inhibition compared to the non-IFN-γ treated cells for that particular experiment. Thus, if a keratinocyte culture had 5×10^5 cells on

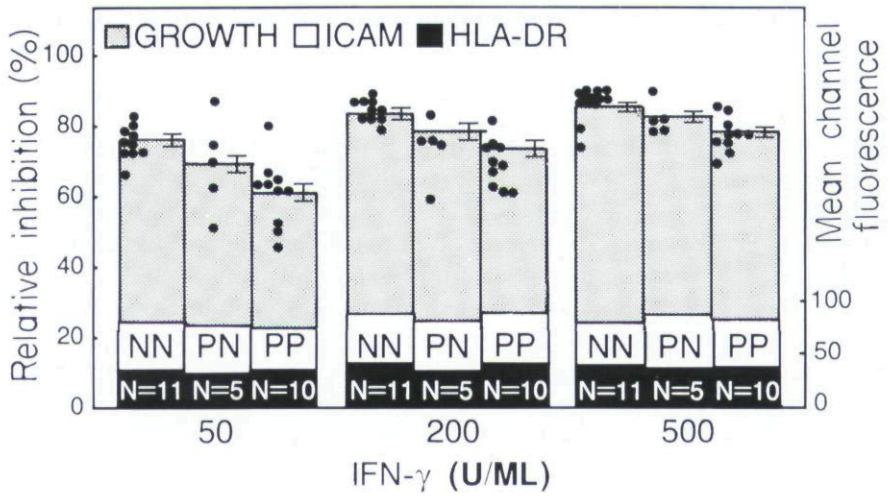

FIGURE 1. The relative growth inhibitory effects and induction of ICAM-1 and HLA-DR by IFN-γ (50, 200 and 500 U/ml) are summarized for multiple passages of all subjects on day 4 as compared to keratinocytes grown in the absence of IFN-γ. The left side vertical axis and stippled bars represent the relative percentage inhibition of growth by IFN-γ on NN, PN, and PP keratinocytes. The right side vertical axis and the open bars (ICAM-1), and bars composed of small boxes (HLA-DR) represent the mean channel fluorescence values of the relative immunomodulatory effects of IFN-γ on NN, PN and PP keratinocytes.

day 4 in the absence of IFN-γ, and had 1×10^5 cells in the presence of IFN-γ, then this would be reported as 80% relative inhibition. The degree of variation in proliferation that we observed is similar to that reported by O'Keefe and Chiu.[30] In contrast to a previous report in which foreskin keratinocytes grew more rapidly in serum containing medium than keratinocytes obtained from normal adult skin,[31] no significant difference in the degree of proliferation between days 0 and 4 was observed in our tissue culture system and therefore the two neonatal foreskin NN keratinocytes were combined with the nine adult NN keratinocyte data.

The relative antiproliferative effects of IFN-γ (50, 200 and 500 U/ml) on passage Nos. 4, 5 or 6 keratinocytes compared to the same keratinocytes grown in the absence of IFN-γ on day 4 is summarized graphically in Figure 1 for all the subjects in this study. The mean relative percentage inhibition on the NN keratinocytes by IFN-γ (50, 200 and 500 U/ml) was 76 ± 2, 84 ± 2 and 88 ± 2 respectively, which included nine adult and two neonatal foreskins and a total of 33 experiments (triplicate experiments on 11 different NN keratinocyte cultures). The mean percentage inhibition on the PP keratinocytes by IFN-γ (50, 200 and 500 U/ml) was 59 ± 2, 69 ± 3 and 79 ± 3 respectively, which included 10 different psoriatic patients. The difference between the NN and PP group mean percentage inhibition for all three IFN-γ concentrations was highly statistically significant ($P < 0.001$). The mean percentage inhibition on the PN keratinocytes by IFN-γ (50, 200 and 500 U/ml) was 68 ± 2, 77 ± 2 and 83 ± 2 respectively which included five different psoriatic patients. The group mean difference between NN and PN keratinocytes was less dramatic than with PP keratinocytes. There was no statistically significant difference between NN and PN keratinocytes at 50 U/ml ($P > 0.1$), but the differences were statistically significant at 200 U/ml ($P < 0.05$) and 500 U/ml ($P < 0.01$).

We next used the NN group mean percentage inhibition by IFN-γ (50, 200 and 500 U/ml) of 76, 84 and 88% respectively, to compare individual PP and PN cultured keratinocytes

antiproliferative responses to IFN-γ. We found that in nine of 10 PP keratinocyte cultures (patients Nos. 1–6 and 8–10) and two of five PN keratinocyte cultures (patients 5 and 10), there was less growth inhibition by IFN-γ ($P < 0.001$ for PP keratinocytes; and $P < 0.01$ for PN keratinocytes vs. NN keratinocytes). The keratinocytes from the atopic and CTCL patients showed a similar antiproliferative response to IFN-γ as the 11 different adult NN keratinocytes (data not shown). This relatively greater proliferation in the presence of IFN-γ for the PP and PN keratinocytes was consistently observed when using keratinocyte cultures between passage No. 2 and passage No. 8.

Morphological appearance of NN and PP keratinocytes

With phase contrast microscopy, there were also morphological differences between NN and PP keratinocytes both in the absence and in the presence of 50 U/ml of IFN-γ. On day 0, the PP keratinocytes appeared slightly larger and less basaloid compared to NN keratinocytes (Fig. 2). On day 4, the 50 U/ml of IFN-γ treated NN keratinocytes revealed numerous foci of stratified clusters of keratinocytes with the adjacent attached cells having a differentiated appearance as previously described.[15] In contrast, the PP keratinocytes on day 4 of IFN-γ (50 U/ml) revealed a greater density of attached cells with only a rare, small cluster of cells, with the adjacent keratinocytes retaining a more basaloid, less differentiated, appearance than the comparable NN keratinocytes (Fig. 2).

Immunomodulatory effects of IFN-γ on NN, PN and PP keratinocytes

The ability of IFN-γ to induce HLA-DR and ICAM-1 expression at all concentrations was the same for PP, PN and NN keratinocytes without any statistically significant difference amongst any normal and psoriatic patients (Fig. 1). Even at 50 U/ml, the PP and PN keratinocytes expressed similar amounts of HLA-DR and ICAM-1 as NN keratinocytes. The increased expression of ICAM-1 over HLA-DR is consistent with our previous observation.[23] Thus, our data indicate a dissociation of the antiproliferative effect of IFN-γ from the immunomodulatory effect (induction of HLA-DR and ICAM-1) for the psoriasis patients.

Basal and IFN-γ inducible TGF-α production by NN and PP keratinocytes

We examined the amount of TGF-α produced by six different NN keratinocyte cultures (NN 4–9) compared to five different PP keratinocyte cultures (PP 6–10) between passages Nos. 2–5, both in the presence and absence of IFN-γ (50 U/ml). Figure 3 reveals that the NN keratinocytes (left panel) produced between 0·4–1·1 ng/ml per 10^5 cells (mean = 0·78 ± 0·29) in the absence of IFN-γ, whereas the PP keratinocytes (right panel) produced between 1·7–5·2 ng/ml per 10^5 cells (mean = 2·78 ± 1·52). The difference in these mean values comparing NN to PP keratinocytes is significant ($P < 0.01$). It should be noted that all of the cultures were seeded at the same density and that the TGF-α measurements were made from 48 h conditioned media (1·5 ml of KGM).

When the NN keratinocytes were exposed to IFN-γ (50 U/ml), they produced an increased amount of TGF-α (approximately 2–3 fold) whereas the PP keratinocytes showed either no response (PP6, 7, 10) or only a slight increase (PP 8, 9). Thus, the PP keratinocytes were much less responsive to the effects of IFN-γ with respect to their increased TGF-α production compared to NN keratinocytes. This increased production of TGF-α was not due to release of cell surface bound TGF-α, since brief treatment of thoroughly washed NN and PP keratinocytes with 'cold acid', which removes cell surface molecules,[24] revealed similar amounts of TGF-α for both IFN-γ treated and untreated NN and PP keratinocytes which was approximately 10% of the amount detected in the conditioned media (data not shown).

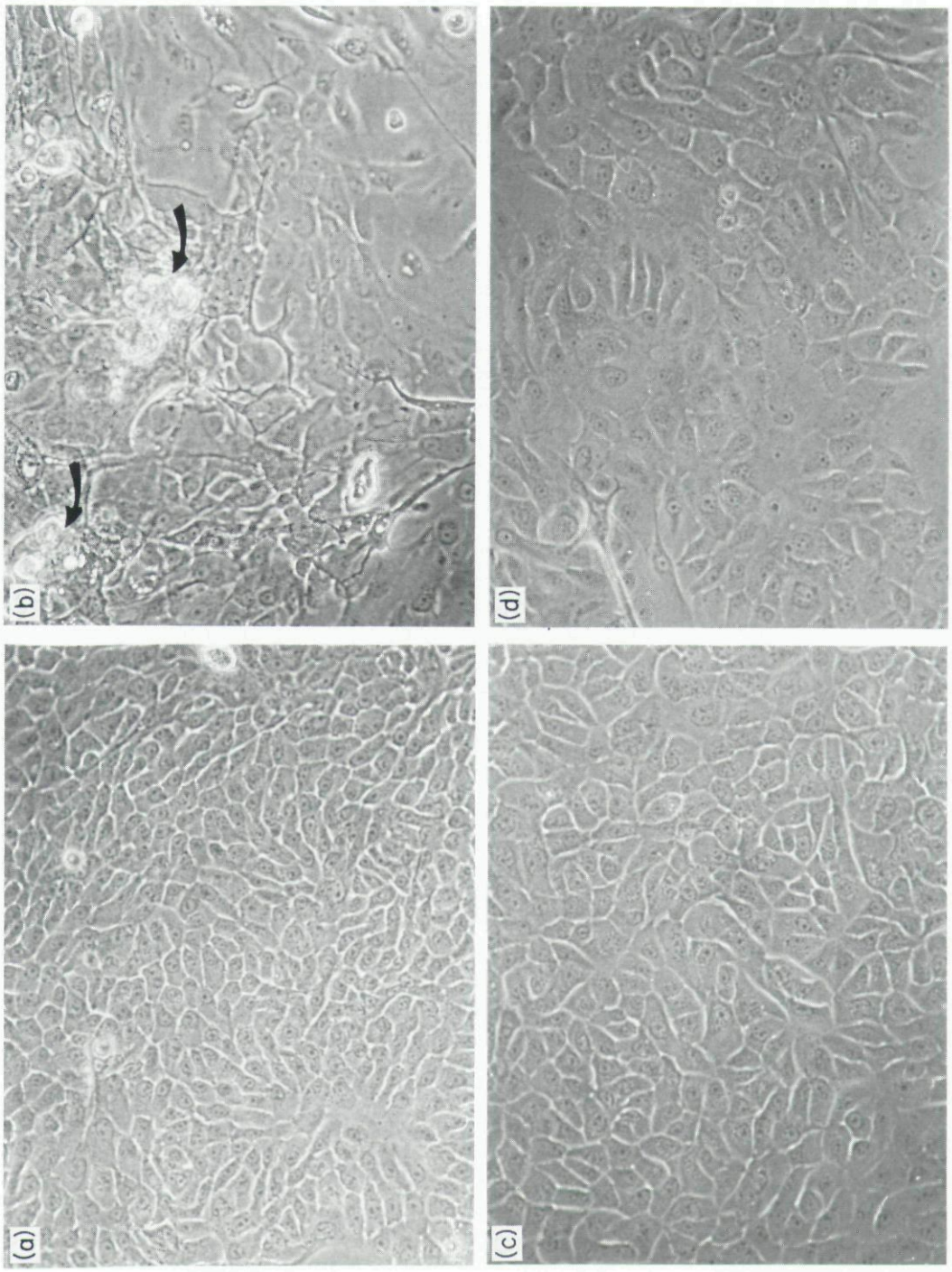

FIGURE 2. Phase contrast microscopic appearance of NN and PP keratinocytes. (a) Untreated NN keratinocytes (passage No. 4). (b) NN keratinocytes 48 h after IFN-γ (50 U/ml). Note the numerous stratified clusters of keratinocytes (arrows). (c) Untreated PP keratinocytes (passage No. 4). Note that compared to NN keratinocytes, the PP keratinocytes are slightly larger, (d) PP keratinocytes 48 h after IFN-γ (50 U/ml). Note the lack of stratified clusters compared to NN keratinocytes ($\times$ 200 mag).

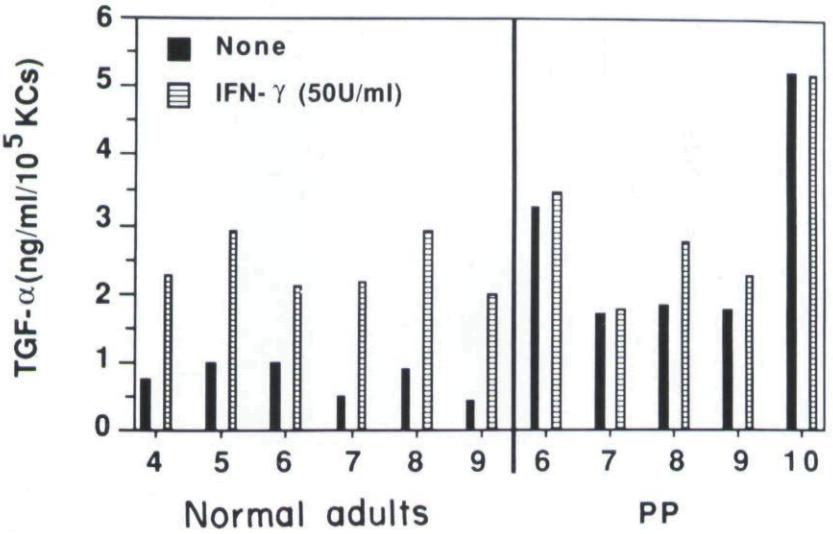

FIGURE 3. The production of TGF-α by six different NN keratinocyte cultures and five different PP keratinocyte cultured with and without IFN-γ treatment resulted in 2–3 fold increases in TGF-α production, whereas there was minimal change in the PP keratinocytes with respect to IFN-γ exposure (SEM < 15%).

The increase in secreted TGF-α protein as detected by the radioimmunoassay of the 48 h conditioned medium, was paralleled by a 3·7 fold stimulation of TGF-α mRNA measured 68 h after IFN-γ treatment of NN keratinocyte cultures (Fig. 4a). Expression of the GAD reference gene varied with time over these extended intervals (Fig. 4b) but displayed little variation in response to IFN-γ (Figs. 4a–b). These results indicate that the increased level of TGF-α secreted in response to IFN-γ reflects increased gene activity as previously reported for the TGF-α gene response to EGF.[22]

DISCUSSION

Using multi-passaged keratinocytes grown on plastic, in a low calcium serum free medium, the PP keratinocytes in nine of 10 patients grew significantly better in the presence of IFN-γ, compared to either NN keratinocytes, or keratinocytes obtained from atopic and Sézary syndrome patients. Our tissue culture system is different from previous investigations which relied on primary cultures of epidermal cells, or keratinocytes grown in serum containing medium in the presence of feeder layers or collagen.[4–9] Utilization of passage Nos. 2–8 keratinocytes reduces possible effects of various diffusible growth modulating factors, which are derived from other cellular constituents in the psoriatic plaque, and retained by primary cultures of keratinocytes. The greater relative proliferation in the presence of IFN-γ which occurred in nine of 10 PP cultures and in only two of five PN cultures may reflect heterogeneity, due to either the type of keratinocyte isolated from the psoriatic plaque,[32] or isolation of different basal cell subpopulations having different *in vitro* responsiveness.[33] Our results in which differences were observed throughout multiple generations of keratinocytes raises the possibility of a genetically transmissable alteration in PP keratinocytes. This *in vitro* observation

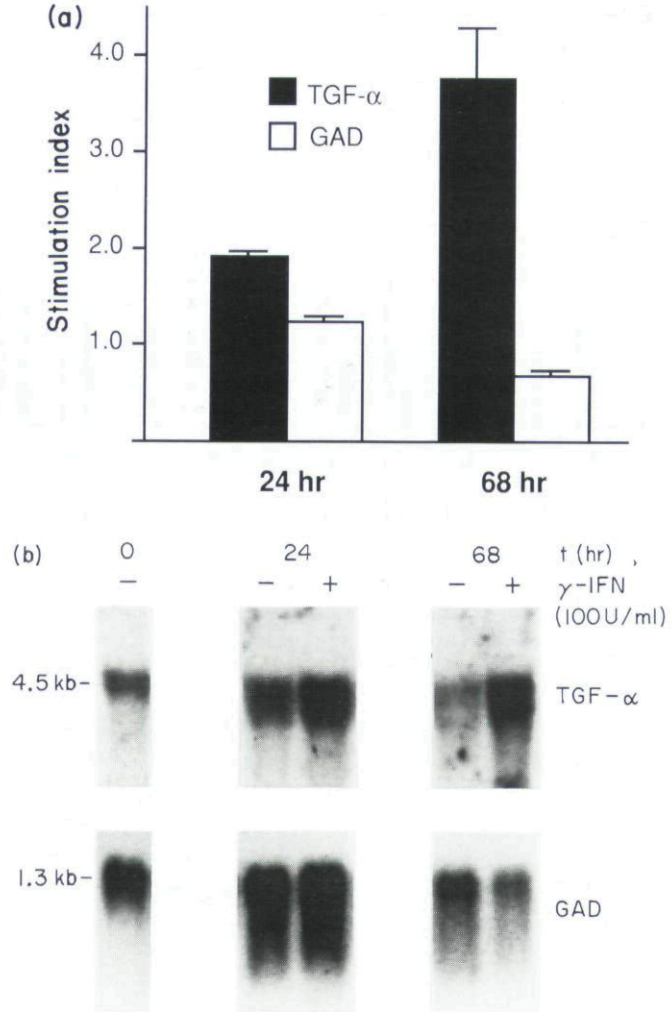

FIGURE 4. RNA blot hybridization analysis of effects of IFN-γ treatment of TGF-α mRNA levels in NN keratinocytes. (a) Densitometric analysis. Integrated autoradiographic intensities are presented as a stimulation index, defined as the ratio of the intensity in the presence of IFN-γ (100 U/ml) to that in its absence at 24 and 68 h after treatment. Results shown are the mean and range of duplicate determinations from a representative experiment. (b) Autoradiography: Only the relevant bands are shown. Upper panel, TGF-α probe. Lower panel, GAD probe. Molecular weights of the hybridizing bands are shown to the left.

is consistent with clinical and epidemiological data suggesting that genetic factors play an aetiological role in psoriasis.[34,35]

Since differences in response to IFN-γ were much more pronounced in PP than PN skin (which may have been involved in psoriasis at some time in the past), it suggests that this difference in behaviour of the keratinocytes may have been produced during the psoriatic process. It should be noted that Baker *et al.* have recently observed that keratinocytes obtained from psoriatic lesions are also less growth inhibited by IFN-γ than normal keratinocytes.[36]

They also report however, that HLA-DR expression by the psoriatic keratinocytes was less after IFN-γ exposure.[36] We currently have no explanation for this difference. Our inability to detect any difference in the ability of IFN-γ to induce HLA-DR or ICAM-1 despite the decreased antiproliferative effect on PP keratinocytes, suggests a dissociation between the immunomodulatory and antiproliferative effects in these cells.

One possible explanation for the apparent refractoriness of PP epidermis to the effects of IFN-γ involves TGF-α and its cellular receptor, the epidermal growth factor (EGF) receptor. IFN-γ-induced HLA-DR expression in cultured human thyroid epithelial cells can be suppressed by TGF-α and its evolutionary and functional homologue,[22] EGF.[19] We have recently observed that the TGF-α gene is overexpressed at the levels of mRNA and protein in psoriatic epidermis, whereas expression of the EGF gene was undetectable.[21] Increased EGF receptor expression by keratinocytes in psoriatic epidermis has been previously demonstrated by Nanney *et al.*[37] These results suggest that TGF-α-mediated signal transduction through the EGF receptor is a prominent feature of psoriatic epidermis. By analogy to thyroid epithelial cells, EGF receptor activation by TGF-α may block the ability of IFN-γ known to be present in psoriatic lesions to induce HLA-DR expression and inhibit proliferation. Our conditions of cell culture provide both normal and psoriatic keratinocytes access to saturating amounts of EGF, while keratinocytes in psoriatic lesions *in vivo* are exposed to higher levels of TGF-α *in situ* than are the keratinocytes of normal epidermis. This difference could explain the lack of differential HLA-DR responsiveness to IFN-γ in PP relative to NN keratinocytes. It is interesting that a very similar dissociation of IFN-γ effects on proliferation and HLA-DR expression has also been reported in human breast cancer cell lines, which, like keratinocytes, display EGF receptors and proliferate in response to EGF.[38] However, the presence of comparable amounts of exogenous EGF cannot explain why PP keratinocytes produced greater amounts of TGF-α than NN keratinocytes in the absence of IFN-γ.

An unexpected linkage between IFN-γ and TGF-α synthesis was discovered during this work. When NN keratinocytes were treated with IFN-γ, there was a significant increase in the amount of TGF-α produced. This increase appeared to reflect increased TGF-α gene activity, as a roughly proportional increase in TGF-α mRNA was observed in NN keratinocytes over the same time interval. In contrast, PP keratinocytes did not further increase their elevated basal TGF-α production in response to IFN-γ. Since IFN-γ is not present in our culture medium and is not produced by keratinocytes, these differences cannot be due to the presence of endogenous IFN-γ under basal conditions. We suspect that psoriatic keratinocytes possess an altered mechanism of signal transduction *in vivo* which persists under *in vitro* culture conditions, increasing basal levels of TGF-α production and inhibiting keratinocyte IFN-γ responsiveness. While the biochemical nature of this defect remains unclear, it could involve increased EGF receptor responsiveness resulting in enhanced TGF-α autoinduction.[21,22]

It is possible that spatial variation in the distribution of IFN-γ responsive vs. IFN-γ unresponsive cells may explain several features of psoriatic lesions. The lesions in psoriasis have been known to be heterogeneous both clinically[32] and histologically.[39] While some of this heterogeneity reflects the presence of epidermal appendages such as hair follicles and eccrine units, a significant contribution is also provided by local variation in the degree of keratinocyte differentiation. We speculate that keratinocytes within a lesion which contain a prominent granular cell layer and absent mitotic figures, including those of follicular epithelium,[40] are IFN-γ responsive and undergoing cellular differentiation.[15] One response of differentiated keratinocytes *in vivo* appears to be a reduction in the number of EGF receptors.[38] In addition to increased production of TGF-α, we have recently shown that IFN-γ causes a down-modulation

of high affinity receptors of EGF in cultured NN keratinocytes.[41] The down modulation of the EGF receptors by IFN-γ may effectively remove a cellular mechanism responsible for negative feedback control of TGF-α production. Thus, IFN-γ responsive keratinocytes may serve as a paracrine source of IFN-γ-inducible TGF-α and yet not proliferate in response to it due to a lack of EGF receptors. In contrast, adjacent or neighbouring keratinocytes which are IFN-γ unresponsive, continue to express EGF receptors and therefore, could be driven to hyperproliferate by this induced TGF-α production from IFN-γ responsive keratinocytes.

This proposition represents a novel pathophysiological model which integrates the morphologic heterogeneity known to be present within psoriatic lesions both clinically[32] and histologically,[39] with the growth factor/growth factor receptor[20-22,37] and immunological alterations[12,42] also observed in psoriasis.

If one role of IFN-γ production by activated T lymphocytes is to function as a feedback growth inhibitor [17] regulating keratinocyte proliferation, then decreased responsiveness of the PP keratinocyte to IFN-γ may contribute to the epidermal hyperplasia so characteristic of psoriasis.[1,2] Since PP keratinocytes tended to be more growth inhibited by very high concentrations of IFN-γ (500 U/ml),[43] treating psoriasis patients with exogeneously administered IFN-γ may be therapeutically beneficial.[44] However IFN-γ promotes T lymphocyte and monocyte adherence to keratinocytes,[45] and may increase the trafficking of inflammatory cells into the psoriatic lesion,[46] and this effect may counteract its direct antiproliferative effect[14] and thus not produce any 'net' clinical response.[47] The observed lack of inhibition of PP keratinocyte proliferation *in vivo* following intralesional IFN-γ administration, despite induction of keratinocyte HLA-DR expression[47] is consistent with our current results. In patients with lepromatous leprosy, intradermal injection of IFN-γ induced a dermal inflammatory reaction accompanied by epidermal hyperplasia and keratinocyte HLA-DR expression.[48] This result also suggests that, *in vivo*, there may be a dissociation of the antiproliferative effects of IFN-γ from its immunomodulatory role. Further work aimed at understanding the cellular basis for the dissociation of the antiproliferative action from the immunomodulatory action of IFN-γ in psoriasis, as well as a greater appreciation for the diversity of functional responsiveness to IFN-γ expression of EGF receptors and TGF-α production by keratinocytes within psoriatic lesions, may also provide new insights into the pathophysiology of psoriasis.

ACKNOWLEDGMENTS

This was supported in part by a NIH grant AR38957 to Dr B.J.Nickoloff, the Babcock Dermatologic Endowment Fund and the National Psoriasis Foundation.

The authors wish to thank Mr T.Hamilton for statistical analysis.

REFERENCES

1 Weinstein GD, Frost P. Abnormal cell proliferation in psoriasis. *J Invest Dermatol* 1968; **50:** 254–9.

2 Krueger GG, Bergstresser PR, Lowe NJ *et al.* Psoriasis. *J Am Acad Dermatol* 1984; **11:** 937–47.

3 Nickoloff BJ. New strategies for psoriasis therapy. *Cutis* 1986; **37:** 421–3.

4 Harper RA, Rispler J, Urbanek WR. DNA synthesis among uninvolved and involved psoriatic epidermal cells and normal epidermal cells *in vitro*. *J Invest Dermatol* 1978; **70:** 254–6.

5 Saiag P, Coulomb B, Lebreton C *et al.* Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model *in vitro*. *Science* 1985; **230:** 669–72.

6 Davison P, Liu SC, Karasek M. Limitations in the use of ^{3}H-thymidine incorporation into DNA as an indicator of epidermal keratinocyte proliferation *in vitro*. *Cell Tissue Kinet* 1979; **12:** 605–15.

7 Kragballe K, Desjarlais L, Marcelo CL. Increased DNA synthesis of uninvolved psoriatic epidermis is maintained *in vitro*. *Br J Dermatol* 1985; **112:** 263–70.

8 Flaxman BA, Chopra DP. Cell cycle of normal and psoriatic epidermis *in vitro*. *J Invest Dermatol* 1972; **59:** 102–5.

9 Chopra DP, Flaxman BA. Comparative proliferative kinetics of cells from normal human epidermis and benign epidermal hyperplasia (psoriasis) *in vitro*. *Cell Tissue Kinet* 1974; **7:** 69–75.

10 Baden HP, Kubilus J, MacDonald MJ. Normal and psoriatic keratinocytes and fibroblasts compared in culture. *J Invest Dermatol* 1981; **76:** 53–5.

11 Liu SCC, Parsons CS. Serial cultivation of epidermal keratinocytes from psoriatic plaques. *J Invest Dermatol* 1983; **81:** 54–61.

12 Nickoloff BJ. Lymphocyte–keratinocyte interactions mediated through interferon: New observations relevant to psoriasis. *Cutis* 1984; **34:** 445–6.

13 Basham TY, Nickoloff BJ, Merigan TC, Morhenn VB. Recombinant gamma interferon induces HLA-DR expression on cultured human keratinocytes. *J Invest Dermatol* 1984; **83:** 88–92.

14 Nickoloff BJ, Basham TY, Merigan TC, Morhenn VB. Antiproliferative effects of recombinant alpha and gamma interferons on cultured human keratinocytes. *Lab Invest* 1984; **51:** 697–701.

15 Nickoloff BJ, Mahrle G, Morhenn VB. Ultrastructural effects of recombinant gamma interferon on cultured human keratinocytes. *Ultrastruct Pathol* 1986; **10:** 17–21.

16 Bjerke JR, Livden JK, Degré M. Interferon in suction blister fluid from psoriatic lesions. *Br J Dermatol* 1983; **108:** 295–9.

17 Zullo JN, Cochran BH, Huang AS, Stiles CD. Platelet derived growth factor and double stranded ribonucleic acids stimulate expression of the same genes in 3T3 cells. *Cell* 1985; **43:** 793–800.

18 Nickoloff BJ. Interferons and Psoriasis—1987 Perspective. *Dermatologica* 1987; **175:** 1–4.

19 Todd I, Pujol-Borrell R, Belfiore A, Bottazzo GF. Thyrocyte HLA class II expression and regulation in relation to thyroid autoimmunity. *Acta Endocrinol (Copenh)* 1987; **281:** (Suppl) 27–34.

20 Gottlieb AB, Chang CK, Posnett DN *et al.* Detection of transforming growth factor α in normal, malignant, and hyperproliferative human keratinocytes. *J Exp Med* 1988; **167:** 670–7.

21 Elder JT, Fisher GJ, Lindguist PB *et al.* Over expression of TGF-α, but not TGF-β, in psoriasis epidermis: Potential role of phospholipase C. *Science* 1989; **243:** 811–4.

22 Coffey RJ, Derynck R, Wilcox JN *et al.* Production and auto-induction of transforming growth factor-α in human keratinocytes. *Nature* 1987; **328:** 817–820.

23 Griffiths CEM, Voorhees JJ, Nickoloff BJ. Gamma interferon induces different keratinocyte cellular patterns of expression of HLA-DR and DQ and intercellular adhesion molecule-1 (ICAM-1) antigens. *Br J Dermatol* 1989; **120:** 1–8.

24 Ascoli M. Internalization and degradation of receptor-bound human choriogonadotrophin in Leydig tumor cells. *J Biol Chem* 1982; **257:** 13306–11.

25 Maniatis T, Fritsch EF, Sambrook J. Extraction, purification and analysis of mRNA from eukaryotic cells. In: *Molecular Cloning, a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982; 188–209.

26 Thompson CB, Challoner PB, Neiman PE, Groudine M. Levels of *c-myc* oncogene mRNA are invariant throughout the cell cycle. *Nature* 1985; **314:** 363–6.

27 Feinberg AP, Volgelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1983; **132:** 6–13.

28 Gupta AK, Fisher GJ, Elder JT *et al.* Sphingosine inhibits phorbol ester-induced inflammation, ornithine decarboxylase activity, and activation of protein kinase C in mouse skin. *J Invest Dermatol* 1988; **11:** 486–91.

29 Dugaiczyk A, Haron JA, Stone EM *et al.* Cloning and sequencing of a deoxyribonucleic acid copy of glyceraldehyde 3-phosphate dehydrogenase messenger ribonucleic acid isolated from chicken muscle. *Biochem* 1983; **22:** 1605–13.

30 O'Keefe EJ, Chiu ML. Stimulation of thymidine incorporation in keratinocytes by insulin, epidermal growth factor, and placental extract: Comparison with cell number to assess growth. *J Invest Dermatol* 1988; **90:** 2–7.

31 Gilchrist BA. *In vitro* assessment of keratinocyte aging. *J Invest Dermatol* 1983; **81:** 184s–9s.

32 Griffin TD, Lattanand A, VanScott EJ. Clinical and histologic heterogeneity of psoriatic plaques: therapeutic relevance. *Arch Dermatol* 1988; **124:** 216–20.

33 Barrandon Y, Green H. Three cloncal types of keratinocyte with different capacities for multiplication. *Proc Natl Acad Sci (USA)* 1987; **84:** 2302–6.

34 Farber EM, Nall ML. The natural history of psoriasis in 5600 patients. *Dermatologica* 1974; **148:** 1–18.

35 Farber EM, Nall ML, Watson W. Natural history of psoriasis in 61 twin pairs. *Arch Dermatol* 1974; **109:** 207–11.

36 Baker BS, Powles AV, Valdimarsson H *et al.* An altered response by psoriatic keratinocytes to gamma interferon. *Scand J Immunol* 1988; **28:** 735–40.

37 Nanney LB, Stoscheck CM, Magid M, King LE. Altered ^{125}I-epidermal growth factor binding and receptor distribution in psoriasis. *J Invest Dermatol* 1986; **86:** 260–5.

38 Gastl G, Marth C, Leiter E *et al.* Effects of human recombinant α_2-interferon and γ-interferon on human breast cancer cell lines: Dissociation of antiproliferative activity and induction of HLA-DR antigen expression. *Canc Res* 1985; **45:** 2957–61.

39 Cox AJ, Watson W. Histologic variations in lesions of psoriasis. *Arch Dermatol* 1972; **106:** 503–603.

40 Headington JT, Gupta AK, Goldfarb MT, Voorhees JJ. Downsized follicles and sebaceous gland atrophy characterize psoriasis of the scalp: New findings after morphometric studies. *J Cut Path* 1988; **15:** 313.

41 Nickoloff BJ, Mitra RS. Pretreatment of keratinocytes with gamma interferon dramatically reduces binding by epidermal growth factor. *Clin Res* 1988; **36:** 908.

42 Valdimarsson H, Baker BS, Jonsdottir I, Fry L. Psoriasis: A disease of abnormal keratinocyte proliferation induced by T lymphocytes. *Immunol Today* 1987; **7:** 256–9.

43 Morhenn VB, Nickoloff BJ. The effect of gamma interferon on psoriatic keratinocytes. In: *Proc IV Int. Symp Psoriasis.* (Farber EM, Nall L, Morhenn VB, Jacobs PH, eds), NY: Elsevier, 1987; 355.

44 Morhenn VB, Pregerson-Rodan K, Mullen RH *et al.* Use of recombinant interferon gamma administered intramuscularly for the treatment of psoriasis. *Arch Dermatol* 1987; **123:** 1633–7.

45 Nickoloff BJ, Lewinsohn D, Butcher E *et al.* Recombinant gamma interferon increases binding of peripheral blood mononuclear leukocytes and a Leu3$^+$ T lymphocyte clone to cultured keratinocytes and to a malignant cutaneous squamous carcinoma cell line that is blocked by antibody against the LFA-1 molecule. *J Invest Dermatol* 1988; **90:** 17–22.

46 Nickoloff BJ. Role of interferon gamma in cutaneous trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. *Arch Dermatol* 1988; **124:** 1835–43.

47 Schulze HJ, Mahrle G. Effect of interferons (rIFN-alpha 2, rIFN-gamma) on DNA synthesis and HLA-DR expression in psoriasis. *Arch Dermatol Res* 1986; **278:** 416–18.

48 Kaplan G, Nusrat A, Sarno EN *et al.* Cellular response to the intradermal injection of recombinant human γ-interferon in lepromatous leprosy patients. *Am J Pathol* 1987; **128:** 345–53.