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Modulation of Keratinocyte-derived Interleukin-8 Which Is Chemotactic for Neutrophils and T Lymphocytes

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Interactions between T lymphocytes, neutrophils, and epidermal cells are believed to play a central role in the pathophysiology of psoriasis and other inflammatory cutaneous disorders. Although there is strong evidence that lymphocyte-function–associated antigen-1 (LFA-1) positive T cells are retained in the epidermis via intercellular adhesion molecule-1 (ICAM-1) expression induced on keratinocytes, the molecular basis for the directed migration of T cells or neutrophils towards the epidermis is not known. To investigate whether epidermal keratinocyte-derived products may be important in the migration of T cells and neutrophils into the epidermis, human keratinocytes were cultured in the presence of various cytokines and chemotactic activity of the supernatants were assessed. TNF-α stimulation produced directed migrational responses for both neutrophils and T-lymphocytes (both CD4 and CD8), but not B lymphocytes; 69% of T-cell movement and 80% of neutrophil migration induced by the TNF-α treated keratinocyte cell supernatants could be inhibited by anti-interleukin-8 (IL-8) serum. Using the same antibody, IL-8 was immunoprecipitated from the supernatants of TNF-stimulated ^{35}S-labelled keratinocytes, and a single 7-kd band product detected by SDS-PAGE. In keeping with these biological activities and protein data, Northern blot analysis of total cellular RNA extracted from keratinocyte monolayers hybridized with a ^{32}P-labelled 1-kb cDNA to IL-8 mRNA, revealed induction of the IL-8 gene in the presence of TNF-α and IL-1β, but not IFN-γ. The protein kinase C agonist, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a known stimulator of psoriasiform cutaneous inflammation when applied directly to murine epidermis, strongly induced keratinocyte elaboration of IL-8 mRNA. These studies demonstrate that activated human keratinocytes are capable of producing biologically active IL-8, and provide evidence that keratinocytes can play a key role in mediating the influx of T cells and neutrophils into the epidermis. (Am J Pathol 1991, 139:869–876)

Normal human epidermis is comprised of three main cell types: keratinocytes, which form 95% of all epidermal cells, melanocytes, and Langerhan's cells. Historically, keratinocytes have been believed to function purely in maintaining the structure of the epidermis via their production of cytokeratins, and in the maintenance of a physical barrier to a variety of exogenous injurious stimuli. In contrast, antigen-presenting dendritic Langerhan's cells have been believed to be the sole resident cell type actively involved in epidermal immune response, whereas keratinocytes were believed to be merely passive targets for immunologic attack. In recent years however, after the initial observations that keratinocytes are a potent source of IL-1, and that keratinocytes can be induced to express the class II MHC antigens including HLA-DR, it has become clear that keratinocytes may not only actively participate in epidermal immune responses,[1] but may in fact play key initiating roles.[2]

Psoriasis, which affects approximately 3% of the general population, is one particular inflammatory cutaneous disease in which interactions between T-lymphocytes and the epidermis, are believed to be of central pathophysiologic significance.[3,4] Psoriasis is characterized by keratinocyte hyperproliferation and the accumulation of T

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cells and neutrophils in the epidermis. Retention of T cells in the epidermis of psoriatic lesions is believed to be mediated via the interaction of LFA-1 on the surface of the lymphocyte with its ligand, ICAM-1 on keratinocytes.[5,6] ICAM-1 is not constitutively expressed by keratinocytes, but can be induced both *in vitro*[6] and *in vivo*[7] by the T-cell–derived cytokine IFN-γ. *In vitro* adhesion studies demonstrate that IFN-γ permits adherence between T cells and keratinocytes, a reaction that can be inhibited by monoclonal antibodies to either LFA-1 or ICAM-1.[8]

However, these adherence phenomena do not explain the initial directed migration of T cells towards the epidermis. Psoriatic scales and epidermal homogenates from allergic patch-test reaction sites have been found to possess leukocyte chemotactic activities.[9–11] We and others have previously demonstrated that cytokine-activated keratinocytes elaborate mRNA coding for IL-8.[12,13] IL-8 is a small soluble peptide belonging to a super gene family that also includes β-thromboglobulin, platelet factor-4, monocyte chemotaxis, and activating factor, interferon-induced protein-10 and melanoma-growth stimulatory activity.[14] Although initially isolated on the basis of its neutrophil chemotactic and activating properties, IL-8 has also been shown to possess T-cell chemotactic activity.[15]

In this report, we extend the previous keratinocyte mRNA studies of IL-8, by demonstrating correlative patterns between IL-8 mRNA and protein production, which is biosynthesized and secreted by stimulated keratinocytes. Moreover, this IL-8 protein has functional activity since resultant activated keratinocyte supernatants possess both neutrophil and T-cell chemotactic activity; the majority of which (69–80%) could be blocked with anti-IL-8 antibody. Induction of IL-8 mRNA could also be induced by the protein kinase-C agonist, 12-O-tetradecanoyl phorbol-13-acetate (TPA), an agent known to cause psoriasiform inflammation in mice when applied directly to the epidermis.[16] These in-vitro results complement the previous *in vivo* findings of increased mRNA levels in psoriatic keratome specimens/psoriatic epidermal sheets,[17] the immunohistochemical localization of IL-8 to keratinocytes,[17] and the IL-8 bioactivity in psoriatic scales.[18,19]

Methods

Cell Culture

Human keratinocytes were isolated from keratome biopsies of normal skin and grown as a monolayer on the bottom of 10 cm diameter petri dishes at 37°C, 5% CO_2 in a defined serum-free low calcium medium containing bovine pituitary extract, epidermal growth factor, hydrocortisone and insulin (keratinocyte growth medium, KGM: Clonetics Corp., San Diego, CA) as previously described.[13] Second to fifth passaged keratinocytes were used on reaching 80% confluency. Cells were incubated either in KGM medium alone, or with the addition of TNF-α (250 U/ml, Cetus Corp., Emeryville, CA; specific activity = 2.0×10^9 U/ml), IFN-γ: (100 U/ml, Genentech, South San Francisco, CA; specific activity 1.7×10^9 U/mg), or both in combination (same concentrations). IL-1β was purchased from Genzyme Corp (Boston, MA). After 18 hours incubation, supernatants were removed, frozen, and stored at −80°C until needed for chemotaxis studies (see below). For Northern blot analysis, cells were cultured as mentioned with KGM alone, TNF-α, IFN-γ, and in addition IL-1β (1–10 ng/ml) or TPA (2–10 nM), and RNA extracted from the cells at varying times between 0 and 18 hours (see below).

Immunofluorescence Studies

The standard direct immunofluorescence assay was employed to detect lymphocytes bearing specific membrane antigens by treating cells with monoclonal antibodies conjugated to fluorescein (FITC) or phycoerythrin (PE). Simultest monoclonal reagents, anti-Leu-4 FITC + anti-Leu-12 PE (T-cells—CD3, B-cells—CD19), anti-Leu-3 FITC + anti-Leu-2a PE (T helper—CD4, T suppressor—CD8), and control (IgG, FITC + IgG_2 PE), were purchased from Becton-Dickinson (Mountain View, CA). Briefly, IL-8 induced migrating lymphocytes were removed from the bottom of three identically treated membranes (each sample well contained 10^{-9}M rIL-8 or stimulated undiluted keratinocyte supernatants) by use of a cell scraper. These cells were pooled, washed twice in PBS, suspended in PBS-0.1% NaN_3, and incubated with monoclonal antibodies in the dark at 4°C for 30 minutes. Cells were washed twice with cold PBS-0.1% NaN_3, and the cell surface fluorochromes were analyzed using a FACScan Analyzer (Becton-Dickinson, Mountain View, CA), setting the scatter gates on the control lymphocyte fraction. Laser excitation was at 488 nm, and the average logarithmic fluorescence intensity of 6,000 lymphocytes was measured. The cells were also examined microscopically using a Zeiss microscope with a UV source.

Chemotaxis Assays

Human peripheral blood lymphocytes were separated by a three-step method (20) utilizing Ficoll-Hypaque (Pharmacia Inc, Piscataway, NJ) and Sepracell-MN (Sepratech Corp, Oklahoma City, OK) and further depleted of adherent cells by incubation on plastic for 18 hours at

37°C to obtain a 96% pure lymphocyte preparation. Human peripheral blood neutrophils, obtained from the pellet of the whole blood Ficoll-Hypaque step, were separated by 1% dextran sedimentation for 1 hour at room temperature to a final purity of approximately 98%. Chemotaxis was carried out in Neuro Probe 48-well micro chemotaxis chambers as described by Falk, et al. ([21]; neutrophils) and Bacon et al. ([22]; lymphocytes). Purified neutrophil or lymphocyte suspensions were added to the top well of the chamber and permitted to migrate through a 10-um thick, 3-um porosity polycarbonate membrane (Nucleopore, Pleasanton, CA) toward the bottom chamber sample. Neutrophils were incubated for 30 minutes at 37°C in humidified air with 5% CO_2, the neutrophil-associated polycarbonate membrane was removed, nonmigrating cells were wiped off, and the filter was fixed for 10 minutes in absolute methanol. The filter was air dried and stained for 30 minutes in 2% toluidine blue. The number of cells migrating through the membrane were counted in three random 10 mm grids at 400X with the mean ± SEM calculated for triplicate samples. The lymphocytes were incubated for 2 hours under the same conditions as the neutrophils. The migrating lymphocytes were either stained and quantitated, as previously described for the neutrophil-associated polycarbonate membranes, or collected (see immunofluorescence method) and counted by hemocytometer and analyzed by immunofluorescence. All results are presented as normalized values representing the percent of maximum f-Met-Leu-Phe (Sigma Chemical Co., St. Louis, MO) positive control (5×10^{-8}M for lymphocytes and 10^{-9}M for neutrophils), minus the appropriate negative controls.

Metabolic Labeling and Immunoprecipitation

Keratinocytes (approximately 5×10^6 cells) were initially grown as described earlier but before the addition of cytokine, the medium (KGM) was removed, and replaced by specially prepared methionine-free, cysteine-free KGM (Clonetics Corp., San Diego, CA). After 1 hour, ^{35}S-labeled methionine-cysteine mixture (70%:30%) was added (250 uCi/ml) together with TNF-α (250 U/ml), IFN-γ (100 u/ml) or both (same concentrations). At 18 hours, the supernatants were collected, mixed with 1 ul/ml (pepstatin 1 ng/ml) and frozen to −20°C.

When required, samples were thawed and the volume containing 1×10^7 trichloroacetic acid precipitable counts made up to 3 ml with 20 mM Tris, 150 mM NaCl, pH 7.6 (TBS) with 1% Triton-X-100, 0.5% deoxycholate and 0.1% sodium dodecyl sulfate (TBS-TDS). In some experiments 1 ul/ml protease inhibitor (as mentioned earlier) and rabbit anti-IL-8 antiserum at a dilution of 1:50 was added. The polyclonal rabbit anti-human-IL-8 serum reacted with recombinant IL-8 in Western blots (data not shown). Parallel samples were immunoprecipitated in an identical fashion with pre-immune rabbit serum. After overnight immunoprecipitation at 4°C, 0.4 ml of a 10% suspension of protein A/sepharose beads (Pharmacia) was added to each sample, and the mixture was incubated for 2 hours at room temperature. After repeated washing in TBS-TDS, the samples were boiled in 8 M urea, 50 mM Tris-pH 7.4 and 1% SDS and electrophoresed under reducing conditions on a 15% SDS-polyacrylamide gel. The gel was fixed in 30% ethanol and 10% acetic acid, enhanced, and submitted to autoradiography.

Northern Blot Analysis

Keratinocyte monolayers were lysed with 5 ml 5.7M guanidine HCl containing 100 mM potassium acetate, sonicated on ice for 1 minute, precipitated with 100% ethanol, and centrifuged at 15,000 g for 40 minutes at 4°C. Pellets of nucleic acid were resuspended in guanidine/potassium acetate containing 25 mM EDTA, ethanol precipitated and centrifuged as mentioned for two further cycles, including multiple aspirations through a 20-gauge spinal needle to shear DNA molecules. The final pellets were extracted in phenol-chloroform, dissolved in H_2O, loaded on to formaldehyde-denaturing agarose gels, and electroblotted on to nylon membranes (Nytran, Schleicher and Schnell, Kenne, NH). A 1.3-kb cDNA fragment recognizing IL-8 mRNA (1.8kb) was cloned and ^{32}P-labeled by the random primer method as previously reported[23] to a specific activity of 3×10^8 cpm/μg. The nylon membranes were hybridized overnight, washed at high stringency (0.1% SDS, 65°C), and subjected to autoradiography.

Results

Chemotaxis

Chemotaxis responses of human peripheral blood neutrophils and T-lymphocytes to TNF-alpha–stimulated keratinocyte supernatants and human recombinant IL-8 are depicted in Figure 1. Figure 1a and c show the dose-dependent responses of neutrophils and T-lymphocytes to rIL-8, with maximum movement occurring at 10^{-9}M and 5×10^{-10}M, respectively. Unconcentrated TNF-α stimulated keratinocyte supernatants induced directed migration of polymorphonuclear cells and T-lymphocytes. Figures 1b and d reveal a dilutional decrease in chemotactic biological response out to 1:1,000 for both

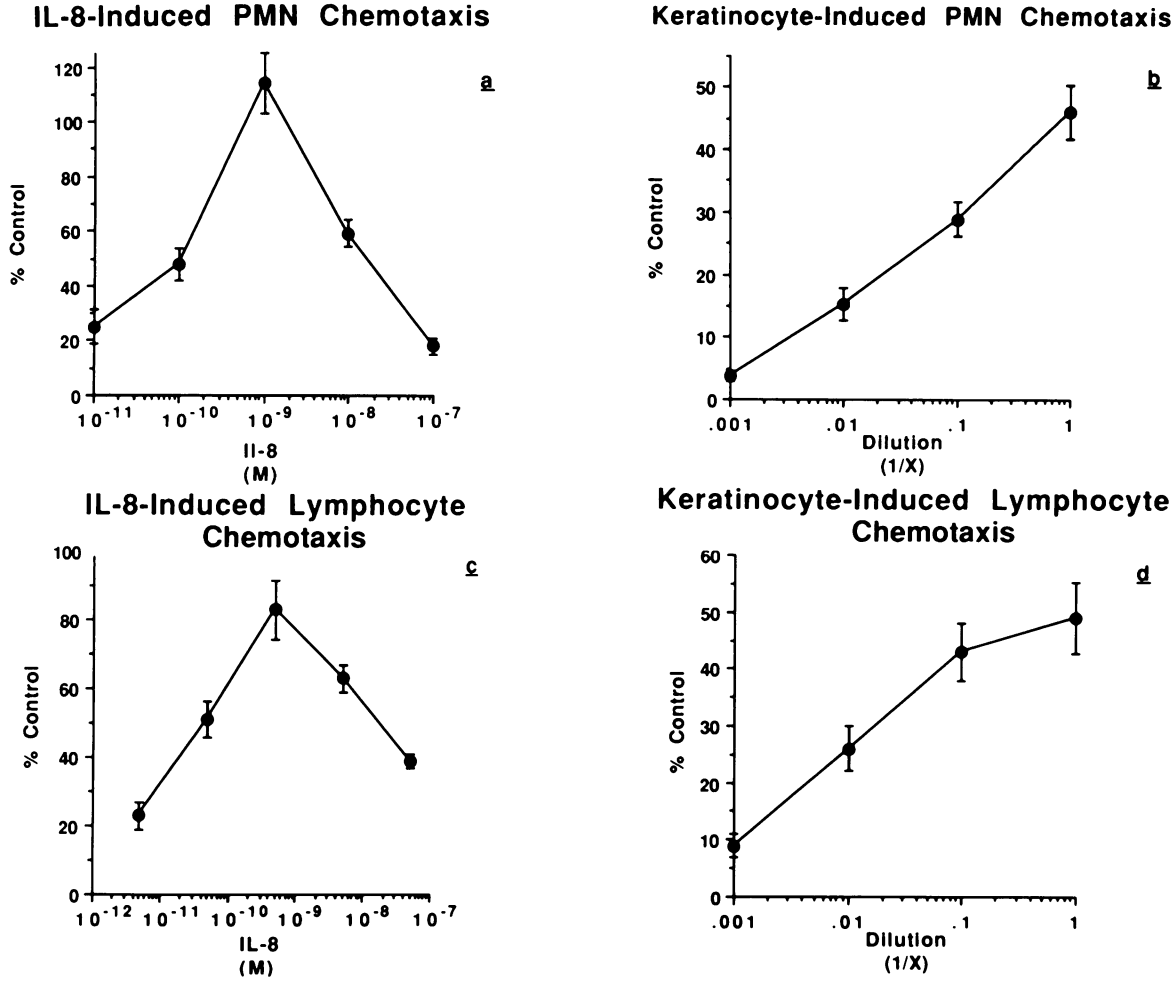

Figure 1. *Chemotactic response of human peripheral blood polymorphonuclear cells (PMN) and lymphocytes to human recombinant IL-8* (a, c) *and TNF-α stimulated keratinocyte supernatants* (b, d). *All data are normalized values representing the percent of maximal chemotactic response to F-Met-Leu-Phe. Background response to buffers used in the assays are subtracted from each value plotted.*

cell types. The maximal response for neutrophils was 46% versus 49% for T-lymphocytes. Keratinocytes stimulated with either TNF-α alone or in combination with IFN-γ, demonstrated up to a six-fold increase in chemotactic activity over their unstimulated or IFN-γ primed counterparts (data not shown), which is in agreement with our immunoprecipitation and IL-8 mRNA data (Figures 2, 3). As previously described,[15] the total number of lymphocytes responding represented only about 7 to 10% of the number added, and the response was not chemokinetic by checkerboard analysis (N = 4).

Anti-IL-8 Antibody Chemotaxis Inhibition

Polyclonal rabbit anti-human-IL-8 serum was found to block up to 69% of T-lymphocyte movement and 80% of neutrophil migration induced by our stimulated keratinocyte supernatants (Table 1). Before chemotactic evaluation, dilutions of the appropriate antisera were incubated with undiluted keratinocyte supernatants for 1 hour at 37°C to allow ample time for neutralization. Specificity of our anti-IL-8 antiserum was supported by a failure to inhibit keratinocyte-induced PMN or T-lymphocyte chemotaxis by using rabbit pre-immune serum. These data indicate the primary neutrophil and T-lymphocyte chemoattractant in stimulated keratinocyte culture supernatants is IL-8.

Characterization of Chemotactic Lymphocytes

Immunofluorescence was used to determine the phenotype of the lymphocytes responding to stimulated keratinocyte supernatants and 10^{-9}M rIL-8. The lymphocyte

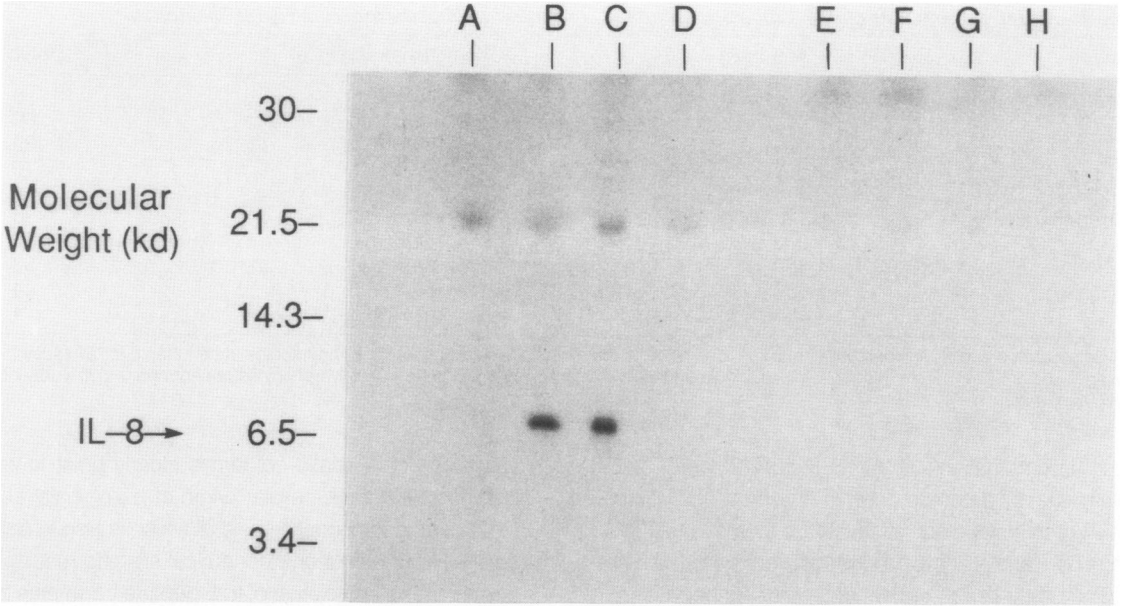

Figure 2. *Immunoprecipitation of IL-8 from cultured keratinocyte supernatants. Lane A: unstimulated cells; Lane B: TNF-α (250 U/ml) alone stimulation; Lane C: TNF-α plus IFN-γ (100 U/ml) stimulation; Lane D: IFN-γ alone stimulation; Lanes E–H: equivalent to Lanes A–D immunoprecipitated with pre-immune rabbit serum.*

population added to the upper well of the chemotaxis chamber was established to have a T-cell/B-cell ratio (Leu-3/Leu-12) of 3.5:1, whereas the T-helper/T-suppressor ratio (Leu-4/Leu-2a) for the migrating population was found to be approximately 3:1. Although the T-helper/suppressor ratio did not change for the responding lymphocytes, the B-cell population does not appear to migrate in response to either the stimulated keratinocyte supernatants or rIL-8. These data suggest IL-8 to be a potent chemoattractant for T-cells, regardless of helper-suppressor phenotype, with little or no ability to induce migration of B-lymphocytes.

Immunoprecipitation of IL-8 from Keratinocyte Supernatants

Under reducing conditions IL-8 was detected as a single protein band of 7kd in the supernatants of ^{35}S-labelled keratinocytes stimulated with TNF-α, alone (Figure 2, lane

Figure 3. *Regulation of IL-8 mRNA production by cultured keratinocytes treated with TNF-α (250 U/ml; 0, 1, 2, 4, 18 hr); TNF-α and IFN-γ (100 U/ml; 18 hours); IFN-γ alone (18 hr) IL-1β (1 ng/ml; 0, 1, 4 hr), and TPA (2.5 nM; 1, 3, 6, 18 hr).*

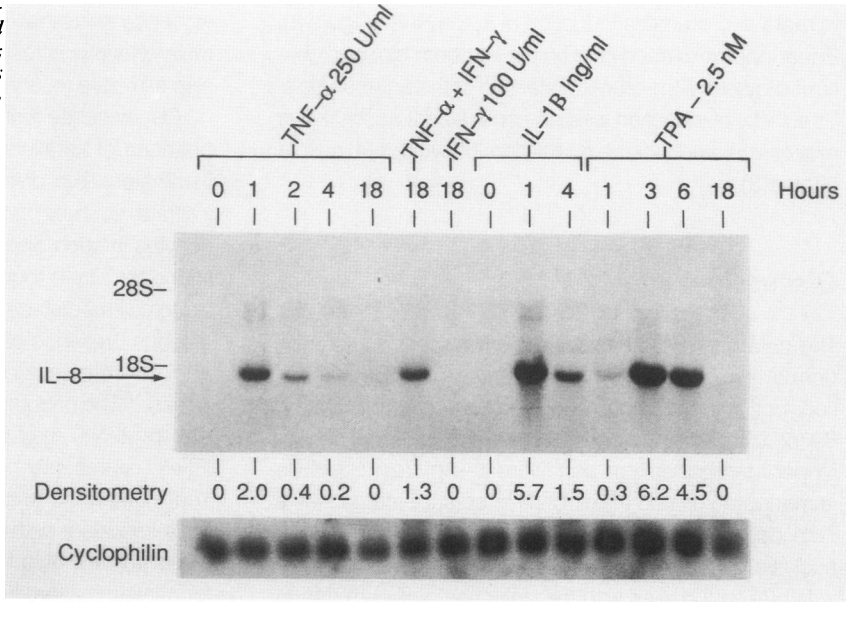

Table 1. *Anti-IL-8 Inhibition of TNF-alpha Stimulated KC-induced Lymphocyte and Neutrophil Chemotaxis*

Cell type	Anti-IL-8	Chemotaxis response*	% Inhibition
Lymphocyte	No antibody	49 ± 6.1	0
	1:200	15 ± 1.8	69
	1:400	30 ± 2.1	39
	1:800	40 ± 4.0	18
	1:1600	47 ± 4.3	4
Neutrophil	No antibody	46 ± 4.3	0
	1:200	9 ± 0.9	80
	1:400	22 ± 3.1	52
	1:800	35 ± 3.3	24
	1:1600	44 ± 4.7	4

* Data represented as the percent of maximal chemotactic response to f-Met-Leu-Phe. The keratinocyte supernatants for all assays were undiluted. The anti-IL-8 serum did not, by itself, influence the chemotactic response of activated lymphocytes or neutrophils in the absence of the KC supernatants.

B), or in combination with IFN-γ (Figure 2, lane C). IL-8 protein was not detected either in unstimulated (Figure 2, lane A) or in IFN-γ alone stimulated supernatants (Figure 2, lane D). Parallel samples immunoprecipitated with preimmune rabbit serum did not detect a 7-kd band (Figure 2, lane E–H).

IL-8 Gene Expression in Cultured Keratinocytes

Consistent with results of chemotaxis and immunoprecipitation studies, IL-8 mRNA was undetectable in nonstimulated keratinocytes but present after TNF-α (250 U/ml) or IL-1β (1 ng/ml) stimulation (Figure 3) as previously described.[2,17] In each case, IL-8 mRNA was rapidly (by 1 hour) but transiently (absent by 8 hours) induced. At no time point examined, did IFN-γ (100 U/ml) induce IL-8 mRNA, although in combination with TNF-α, there was marked persistence of IL-8 gene expression particularly prominent at 18 hours. Incubation with TPA (2.5 nM) led to rapid and dramatic induction of IL-8 mRNA (Figure 3). Equal loading of each lane on the Northern blot was confirmed by ethidium-bromide staining of the agarose gels. Specificity of induction was demonstrated by constitutive expression, and lack of modulation, of cyclophilin mRNA (Figure 3).

Discussion

The data presented here demonstrates that human epidermal keratinocytes, on appropriate cytokine stimulation, possess neutrophil and T-cell chemotactic activity, 69 to 80% of which can be inhibited by anti IL-8 serum. Under identical culture condition, IL-8 protein could be immunoprecipitated from conditioned supernatants as a 7-kd band, and IL-8 mRNA detected by Northern blot analysis of the keratinocyte monolayer. These results, when taken together with the detection of IL-8 mRNA in specimens of psoriatic epidermis clearly point towards keratinocytes as the cellular origin of biologically active IL-8 detected in psoriatic scale,[18] which is also in agreement with our immunohistochemical staining results.[17]

After the initial isolation of IL-8 peptide fragments from psoriatic scale possessing neutrophil chemotactic activity,[19] there has been growing interest in IL-8 as an important mediator in psoriasis. As mentioned earlier, interactions between the epidermis and retained T cells is believed to play a key role in its pathophysiology; IL-8 may well be involved in the attraction of T cells to the epidermis. In the study presented here, B cells did not migrate, whereas the helper:suppressor/cytotoxic (CD4:CD8) ratio of migrating T cells was 3:1. The results are similar to a previous report documenting preferential migration *in vitro* of CD4 positive T cells over CD8 positive T cells in response to epidermal-derived chemotactic factor,[24] and identical to the immunophenotype of the inflammatory cellular infiltrate in psoriasis. Moreover, together with the accumulation of T cells and neutrophils, psoriatic epidermis is characterized by epidermal hyperproliferation and evidence exists that IL-8 in epidermis may explain the two main phenotypic features of psoriasis, because IL-8 can also stimulate keratinocyte growth.[25]

Our evidence further reinforces the concept of the importance of keratinocytes in the pathophysiology of inflammatory skin disease characterized by a T-cell rich infiltrate, such as psoriasis. As stated previously, keratinocytes in such diseases express ICAM-1 an adhesion ligand for T cells together with HLA-DR, a molecule central to cell-mediated responses. Interestingly in the simultaneous presence of TNF-α and IFN-γ, there is marked and coordinate induction of both IL-8 and ICAM-1 genes.[13] The prolongation of TNF-α induced IL-8 expression by IFN-γ may also be relevant to psoriasis since IFN-γ has recently been detected in psoriatic lesions.[26] The marked induction of IL-8 in response to TPA may have important pathophysiologic implications. First, TPA when painted onto the skin of mice produces psoriasiform inflammation.[16] Second, TPA induces cultured ke-

ratinocytes to produce transforming-growth–factor alpha (TGF-α), a potent keratinocyte mitogen that is also elevated in psoriasis[17]; and third, TPA directly induces ICAM-1 in cultured keratinocytes.[27] Thus activation of protein kinase-C signal transduction pathways, may potentially lead to the initiation of cutaneous inflammation and alterations in epidermal growth.

As demonstrated herein, keratinocytes produce IL-8 in response to IL-1β and TNF-α, and both these cytokines may have relevance of epidermal inflammation since both may be produced by keratinocytes. Furthermore, dermal dendrocytes, a population of dermal dendritic cells situated along the dermo-epidermal junction and epidermal Langerhan's cells have been shown to contain immunohistochemically detectable TNF-α in psoriasis.[18] If the dermal dendrocytes and Langerhan's cells were activated in psoriatic lesions,[17] these cells could produce TNF-α and thereby induce the adjacent keratinocytes to produce IL-8. The keratinocyte derived IL-8 could then provide the appropriate T cell and neutrophil chemotactic activity for the intraepidermal recruitment of these leukocytes, which have been linked to the pathophysiology of psoriasis.[3,4]

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References

1. Streilein JW: Skin associated lymphoid tissues (SALT): The next generation. Skin Immune System. Edited by JD Bos. Boca Ratan, CRC Press, 1989, pp 25–48
2. Barker JNWN, Mitra RS, Griffiths CEM, Dixit VM, Nickoloff BJ: Keratinocytes as initiators of inflammation. Lancet 1991, 337:211–214
3. Fry L: Psoriasis. Br J Dermatol 1988, 199:445–461
4. Nickoloff BJ, Griffiths CEM: Lymphocyte trafficking in psoriasis: A new perspective emphasizing the dermal dendrocyte with active dermal recruitment mediated via endothelial cells followed by intra-epidermal T-cell activation. J Invest Dermatol (Suppl) 1990, 93:355–375
5. Nickoloff BJ: Role of gamma interferon in cutaneous trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. Arch Dermatol 1988, 124:1835–1843
6. Griffiths CEM, Voorhees JJ, Nickoloff BJ: 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 1989, 20:617–629
7. Barker JNWN, Allen MH, MacDonald DM: The effect of *in vivo* interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. J Invest Dermatol 1989, 93:439–442
8. Nickoloff BJ, Griffiths CEM, Barker JNWN: The role of adhesion molecules, characteristic factors and cytokines in inflammatory and neoplastic skin disease—1990 update. J Invest Dermatol (suppl) 1990, 94:1515–1575
9. Schroder JM, Christopher E: Identification of C5a des arg and an anionic neutrophil-activating peptide (ANAP) in psoriatic scales. J Invest Dermatol 1986, 87:53–58
10. Camp RDR, Fincham NJ, Cunningham FM, Greaves M, Morris J, Chu A: Psoriatic skin lesions containing biologically active amounts of an interleukin-1 like compound. J Immunol 1986, 137:3469–3474
11. Larsen CG, Ternowitz T, Larsen FG, Thestrup-Pedersen K: Epidermis and lymphocyte interactions during an allergic patch test reaction. Increased activity of ETAF/IL-1, epidermal devried lymphocyte chemotactic factor and mixed skin lymphocyte reactivity in persons with type IV allergy. J Invest Dermatol 1988, 90:230–233
12. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K: Production of interleukin-8 by human derived fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. J Immunol 1989, 68:31–36
13. Barker JNWN, Sarma V, Mitra RS, Dixit VM, Nickoloff BJ: Marked synergism between tumor necrosis factor-alpha and interferon-gamma in regulation of keratinocyte-derived adhesion molecules and chemotactic factor. J Clin Invest 1990, 85:605–608
14. Westwick J, Li SW, Camp RD: Novel neutrophil-stimulating peptides. Immunol Tod 1989, 10:146–147
15. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K: The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. Science 1989, 243:1464–1466
16. Gupta AK, Fisher GJ, Elder JT, Nickoloff BJ, Voorhees JJ: Sphingosine inhibits phorbol ester induced inflammation, ornithine decarboxylase activity, activation of protein kinase C in mouse skin. J Invest Dermatol 1988, 91:486–491
17. Nickoloff BJ, Karabin GD, Barker JNWN, Griffiths CEM, Sarma V, Mitra RS, Elder JT, Kunkel SL, Dixit V: Cellular localization of interleukin-8 and its inducer—tumor necrosis factor-alpha in psoriasis. Am J Pathol 1991, 138:129–140
18. Gearing AJH, Fincham NJ, Bird CR, Madhwa M, Meager A, Cartwright JE, Camp RDR: Cytokines in skin lesions of psoriasis. Cytokine 1990, 2:68–74
19. Schroder JM, Christophers E: Identification of a novel family of highly potent neutrophil chemotactic peptides in psoriatic scales. J Invest Dermatol 1988, 91:395–399
20. Vissers MCM, Jester SA, Fantone JC: Rapid purification of human peripheral blood monocytes by centrifugation through Ficoll-Hypaque and Sepracell-MN. J Immunol Methods 1988, 110:203–207
21. Falk W, Goodwin RH Jr, Leonard EJ: A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. J Immunol Methods 1980, 33:329–335
22. Bacon KB, Camp RDR, Cunningham FM, Wollard PM: Con-

trasting in vitro lymphocyte chemotactic activity of the hydroxyl enantiomers of 12-hydroxy-5,8,10,14-eicosatetraenoic acid. Br J Pharmacol 1988, 95:966–974

23. Dixit VM, Green S, Sarma V, Holzman L, Wolf FW, O'Rouke K, Ward PA, Prochownik EV, Marks RM: Tumor necrosis factor-α induction of novel gene products in human endothelial cells including a macrophage-specific chemotaxin. J Biol Chem 1990, 265:2973–2980
24. Zachariae C, Ternowitz T, Larsen CG, Nielsen V, Thestrup-Pederson K: Epidermal lymphocyte chemotactic factor specifically attracts OKT4-positive lymphocytes. Arch Dermatol Res 1988, 280:354–357
25. Krueger G, Jorgensen C, Miller C, Schroeder H, Stiecherling M, Christophers E: Effects of IL-8 on epidermal proliferation. (abstract) J Invest Dermatol 1990, 94:545
26. Barker JNWN, Karabin G, Stoof T, Mitra RS, Sarma V, Dixit VM, Nickoloff BJ: Detection of gamma interferon mRNA in psoriatic epidermis by polymerase chain reaction. J Dermatol Sci 1991, 2:106–111
27. Griffiths CEM, Esmann J, Fisher GJ, Voorhees JJ, Nickoloff BJ: Differential modulation of keratinocyte intercellular adhesion molecule-1 expression by gamma interferon and phorbol ester: evidence for involvement of protein kinase C signal transduction. Br J Dermatol 1990, 122:333–342