Marked Synergism between Tumor Necrosis Factor-α and Interferon-γ in Regulation of Keratinocyte-derived Adhesion Molecules and Chemotactic Factors

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Abstract

T lymphocytes and mononuclear cells preferentially accumulate in the epidermis in inflammatory skin disease. To determine the role of keratinocytes in both the chemotaxis and adhesion of these cells to the epidermis, cultured keratinocytes were incubated with IFN-γ and tumor necrosis factor-alpha (TNF-α), and mRNA detected and quantitated for IL-8, monocyte chemotaxis and activating factor, and intercellular adhesion molecule-1. Whereas induction of these mRNAs was either absent, or relatively weak and transient, to either IFN-γ or TNF-α alone, when administered in combination there was a dramatic increase and persistence in the induction of all three genes. Pretreatment of the keratinocytes with cycloheximide failed to eliminate transcription, implying that all three are primary response genes. Transforming growth factor-beta, which modulates other keratinocyte functions (not related to adhesion or chemotaxis of inflammatory cells) failed to induce any of the genes. These novel findings potentially explain the selective recruitment of T cells and monocytes observed in inflammatory skin disease, because IFN-γ and TNF-α can coordinately regulate keratinocyte-derived chemoattractants and adhesion molecule production. (*J. Clin. Invest.* 1990. 85:605–608.) epidermis • lymphocytes • macrophages • psoriasis • trafficking

Introduction

Abnormalities of cutaneous lymphocyte trafficking are thought to be central to the pathophysiology of certain chronic inflammatory and neoplastic skin diseases such as psoriasis and cutaneous T cell lymphoma (1). Since T lymphocytes and monocytes, but not B lymphocytes, preferentially accumulate in the epidermis, this implies that trafficking of leukocytes through the skin is nonrandom. In this regard, parallels exist with differential lymphocyte migration in peripheral lymph nodes vs. Peyer's patches (2). That human keratinocytes produce a range of immunoregulatory cytokines (i.e., IL-1, IL-6, and granulocyte/monocyte colony-stimulating factor (3), and can be induced to express intercellular adhesion molecule-1 (ICAM-1)[1] and class II major histocompatibility antigens HLA-DR and HLA-DQ (4, 5), suggests that they may play a fundamental and active role in the immunobiology of the skin. In this study, we sought to determine whether IFN-γ and tumor necrosis factor-alpha (TNF-α), which are themselves soluble products of lymphocytes and monocytes, respectively, regulate keratinocyte elaboration of specific chemotaxins, namely IL-8 (6), monocyte chemotaxis and activating factor (MCAF) (7, 8), and an adhesion molecule, ICAM-1.

Methods

Keratinocyte culture. Human keratinocytes were obtained from fresh keratome biopsies of normal skin and cultured in a serum-free medium containing epidermal growth factor, insulin, and bovine pituitary extract (keratinocyte growth medium; Clonetics Corp., San Diego, CA) at 37°C, 10% CO_2. Cells used in this study were derived from the second to fourth passages grown as a monolayer to subconfluency in 10-cm-diam plastic petri dishes. The cells were then washed and 5 ml fresh medium added to each dish containing recombinant human IFN-γ (100 U/ml; Genentech Inc., South San Francisco, CA), specific activity = 1.7×10^7 U/mg, recombinant human TNF-α (250 U/ml; Cetus Corp., Emeryville, CA), specific activity = 2.0×10^7 U/mg, or both (same concentrations). At times 0, 1/2, 1, 2, 4, 8, and 18 h, the supernatant was discarded and RNA extracted from the keratinocytes. Incubation of keratinocytes with transforming growth factor-beta (TGF-β) (Genentech Inc.) for 4 h was also performed.

RNA preparation procedure. Monolayers of keratinocytes were extracted with guanidine hydrochloride (5.7 M), potassium acetate (100 mM, pH 5), sonicated on ice for 60 s, ethanol precipitated, and centrifuged at 15,000 *g* for 30 min at 4°C. The pellets were resuspended in 1.0 ml of guanidine potassium acetate solution containing 25 mM EDTA, ethanol precipitated, and centrifuged as above, for two complete cycles. The pellets were then dissolved in 20 mM Tris pH 8.0, 0.2% SDS, 10 mM EDTA, and 100 mM sodium chloride, and extracted with phenol-chloroform, precipitated, and dissolved in water. 10 μg total cellular RNA was then separated according to molecular weight on 1% agarose gels containing 8% formaldehyde and electroblotted on nylon membranes (Bio-Rad Laboratories, Richmond, CA).

Northern blot hybridization. cDNA probes for IL-8, MCAF, ICAM-1, and endothelial cell lymphocyte adhesion molecule-1 (ELAM-1) were cloned by differential hybridization as detailed elsewhere (9). Each insert was purified by agarose gel electrophoresis and electroelution and ^{32}P-labeled by the random primer method (10) to a specific activity of 3×10^8 cpm/μg. The nylon membranes were hybridized overnight, washed in 0.3 M sodium chloride, 0.03 M sodium

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Received for publication 3 October 1989.

J. Clin. Invest.

0021-9738/90/02/0605/04 $2.00
Volume 85, February 1990, 605–608

1. *Abbreviations used in this paper:* CHX, cycloheximide; ELAM-1, endothelial cell lymphocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; MCAF, monocyte chemotaxis and activating factor; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha.

citrate, and 0.2% SDS at 65°C, and subjected to autoradiography. cDNA probe for cyclophilin mRNA was included as the reference gene (11).

Quantification of mRNA. The relative density of the signals from each lane of the Northern blots were determined by scanning laser densitometry. The area under the peak corresponding to each hybridized band was divided by the area under the corresponding cyclophilin peak. The results were then expressed as the percentage of the maximum densitometry measurement plotted against time (hours). The results demonstrated are representative of three separate studies.

Results

Time course of the effects of TNF-α and IFN-γ on IL-8 production. Whereas mRNA was undetectable in unstimulated and IFN-γ–treated keratinocytes, TNF-α induced IL-8 gene transcription (Figs. 1 and 2). Keratinocyte response to TNF-α was rapid but transient, with mRNA detectable by 1 h, maximal between 2 and 8 h and absent at 18 h. When TNF-α and IFN-γ were administered simultaneously, IL-8 mRNA levels were considerably elevated. Furthermore, whereas transcription had previously been transient, there was a sustained increase such that high levels of mRNA were still evident at 18 h.

Time course of the effects of TNF-α and IFN-γ on MCAF production. Keratinocytes constitutively expressed low levels of MCAF mRNA which was unaffected by incubation with TNF-α (Figs. 1 and 2). IFN-γ upregulated MCAF mRNA by 4 h, but by 18 h transcription had returned almost to basal levels. In combination, TNF-α and IFN-γ superinduced MCAF mRNA such that transcription was detected by 2 h and present at 18 h and at all times examined was considerably greater than with IFN-γ alone.

Time course of the effects of TNF-α and IFN-γ on ICAM-1 production. ICAM-1 mRNA was not present in unstimulated keratinocytes. TNF-α produced a weak and transient rise in ICAM-1 mRNA between 2 and 8 h. IFN-γ produced a substantial increase in ICAM-1 mRNA which persisted to 18 h. When TNF-α and IFN-γ were administered together, keratinocyte ICAM-1 production was superinduced at all time points studied.

Effects of cycloheximide (CHX) and TGF-β on IL-8, MCAF, and ICAM-1 production. Pretreatment of the keratinocytes with CHX failed to inhibit transcription of each of the three genes examined (Figs. 1 and 2). TGF-β (24 ng/ml) did not induce any of the mRNAs studied (data not shown; although we have previously observed that these concentrations inhibit keratinocyte proliferation and induce keratinocyte motility and extracellular matrix production [12]).

ELAM-1 production by keratinocytes. Keratinocytes failed to elaborate ELAM-1, an adhesion molecule for polymorphonuclear leukocytes (9, 13), at any point examined (data not shown).

Discussion

The inflammatory dermatoses, such as psoriasis and lupus erythematosus, and neoplastic disease, such as cutaneous T cell lymphoma are characterized immunohistochemically by the

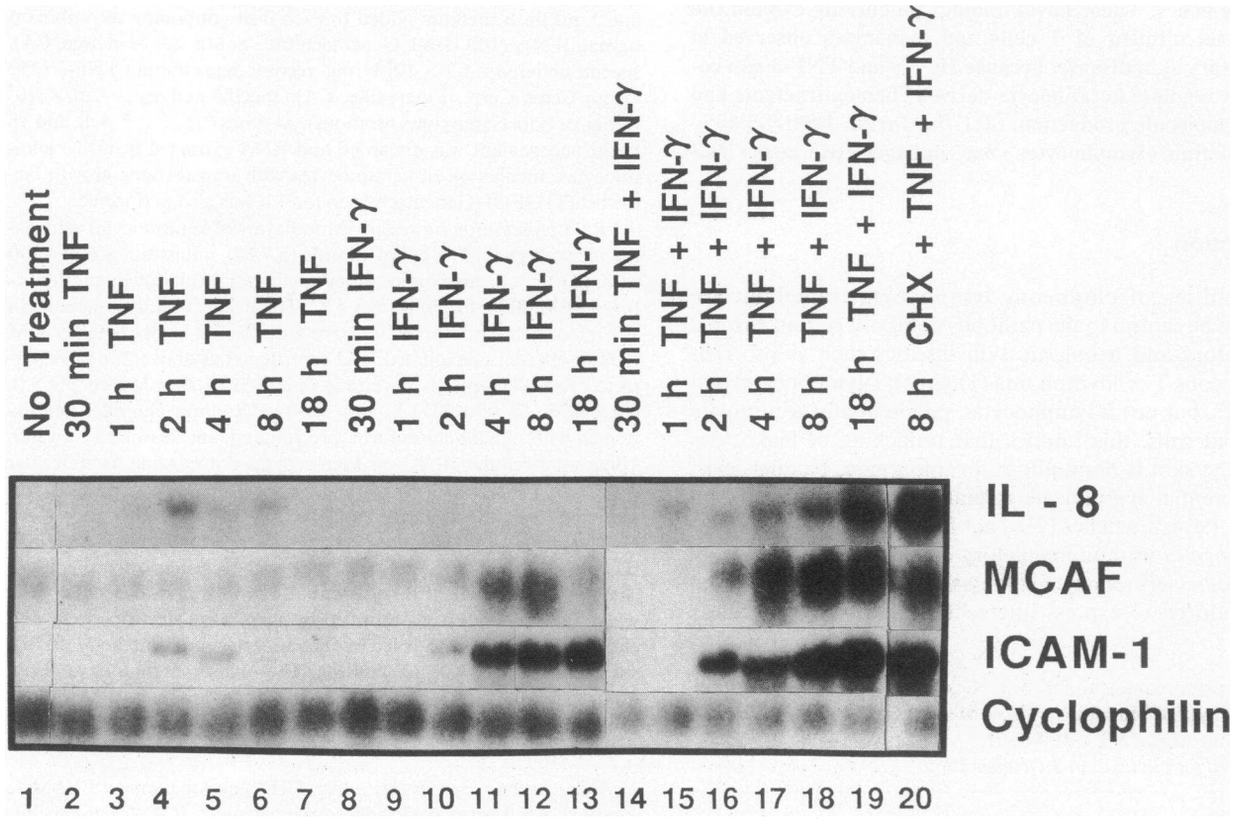

Figure 1. Composite figure of Northern blots obtained from human keratinocytes stimulated with IFN-γ and TNF-α alone and in combination, probed for IL-8, MCAF, and ICAM-1. The bottom track represents one blot reprobed for cyclophilin mRNA. Lane *20* demonstrates the effect of pretreatment with CHX (10 μg/ml) on the stimulated keratinocytes at 8 h.

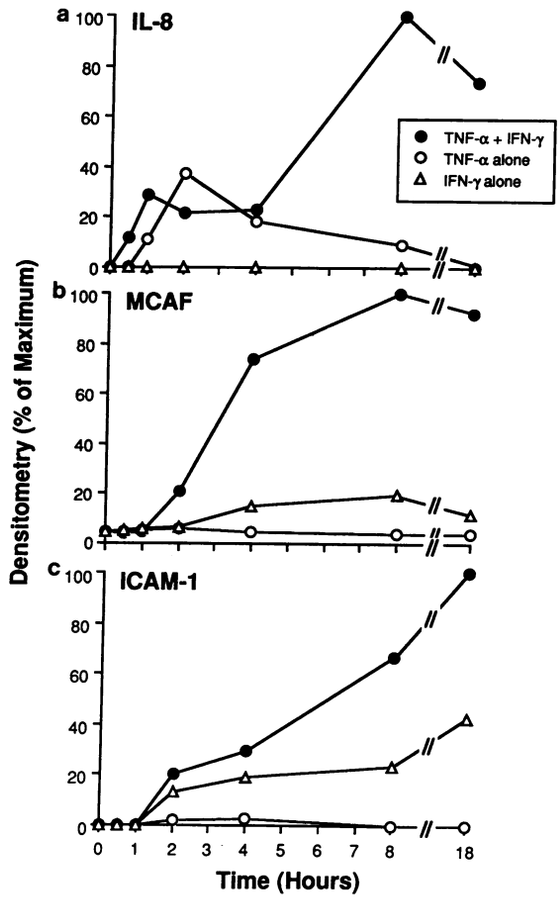

Figure 2. Relative density of the signals from each lane of the Northern blots (*a*) IL-8, (*b*) MCAF, and (*c*) ICAM-1, as determined by scanning laser densitometry.

presence of monocytes and activated T lymphocytes within the epidermis. Although in vitro studies suggest that epidermal trapping of these mononuclear cells is mediated via lymphocyte function associated antigen-1/ICAM-1 interactions (1), the mechanisms whereby the cells leave the perivascular compartment in the dermis and migrate to the epidermis remain unclear. Studies that demonstrated that epidermis overlying positive dermal tuberculin reactions possess chemotactic properties have not provided either a molecular basis for this reaction or determination of the initiating cell type (14). Of particular importance at present is elucidation of the chemotaxins responsible for the attraction of T lymphocyte and monocytes toward the epidermis. The fact that these cell types preferentially accumulate in the epidermis suggests that the epidermis itself elaborates specific chemotaxins. Keratinocyte-derived IL-8 (15) may be involved in T cell chemotaxis and our studies provide the first evidence that keratinocytes are a source of MCAF. It is thus possible to envisage a model of cutaneous inflammation in which the coordinate induction of specific chemoattractant and adhesion molecules by keratinocytes plays a vital role.

Circulating inflammatory cells interact with endothelial cells which results in their sequestration into the perivascular dermis (16). Since there is close spatial apposition between dermal vessels and the epidermis (17), release of cytokines by the infiltrating cells would interact with the epidermis to induce keratinocyte activation. Two important cytokines in this respect are IFN-γ derived from activated T cells and TNF-α derived from monocytes, both of which interact with keratinocytes via specific surface receptors (18, 19). As we have demonstrated here, these cytokines induce keratinocytes to elaborate specific chemotaxins for these same cell types. Once in the epidermis direct adherence between keratinocytes and the infiltrating mononuclear cells occurs via ICAM-1/lymphocyte function associated antigen-1 interactions, the consequences of which are thought to be central to the pathophysiology of inflammatory skin disease (1). Such a scenario may explain the persistence of cutaneous inflammation in that keratinocyte/mononuclear cell interactions generate further chemotactic, adhesion, and proinflammatory molecules. Furthermore, the presence of mononuclear cells within the dermal compartment are not essential for the initial development of the epidermal inflammation, since some agents known to produce cutaneous inflammation such as phorbol esters, which activate protein kinase C, are direct inducers of both keratinocyte ICAM-1 expression (20) and IL-8 production (J. N. W. N. Barker, V. Sarma, V. M. Dixit, and B. J. Nickoloff, unpublished observation) in the absence of mononuclear cells or other cytokines. This primary alteration in keratinocyte signal transduction involving protein kinase C, which can be induced via a host of endogenous as well as exogenous stimuli (17), could primarily initiate the influx and retention of inflammatory cells via chemotactic factors and adhesion molecules.

One of the main functions of the skin is to act as a barrier to external injurious stimuli such as trauma, whether physical or chemical, virus or bacterium. Cutaneous responses to such stimuli requires the rapid induction of inflammation which should, in the most part, be transient so that the inflammatory process is downregulated in a timely fashion after elimination of the precipitating agent. Such intrinsic regulation by the keratinocytes in response to low levels of TNF-α and IFN-γ appear to be preserved in our culture system because of the transient IL-8 response to TNF-α and MCAF response to IFN-γ (Fig. 2). However, if the stimulus is sufficiently strong, or persistent, high enough tissue levels of IFN-γ and TNF-α may be generated by the T cells and monocytes, respectively, within the inflammatory infiltrate to trigger a more chronic response as seen with the synergistic effects of TNF-α and IFN-γ (Fig. 2). Also, it is interesting to speculate that certain inflammatory skin diseases, such as psoriasis, may arise secondary to the inappropriate persistence of mononuclear cells within the epidermis due to the misregulation of keratinocyte-elaborated chemotactic and adhesion molecules such as IL-8, MCAF, and ICAM-1 (21). Central to these concepts are the coordinate regulation of chemotactic and adhesion molecules. As demonstrated here, neither IFN-γ nor TNF-α alone induces activation of all three genes, and TGF-β, which appears to influence other aspects of keratinocyte behavior having more to do with mitosis, matrix production, and migration (12), does not induce any of these genes. On an experimental level, this implies that the activity of one cytokine may be altered when other molecules are added, which presumably mirrors more closely events in vivo.

Acknowledgments

The authors wish to thank Dr. J. T. Elder for his assistance regarding cyclophilin mRNA.

This work was supported in part by the British Association of Dermatologists Dowling Travelling Fellowship (Dr. Barker), the Psoriasis Association of Great Britain (Dr. Barker), and the National Institutes of Health (AR-38957, 40065, and 01823 [Dr. Nickoloff], and NHLBI 39415 [Dr. Dixit]).

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