Cytokine-Induced ICAM-1 Expression in Human Keratinocytes Is Highly Variable in Keratinocyte Strains from Different Donors

Marjorie H. Middleton and David A. Norris

Department of Dermatology, University of Colorado School of Medicine; and Department of Veterans Affairs Hospital, Denver, Colorado, U.S.A.

Induction of intercellular adhesion molecule 1 (ICAM-1) expression in the epidermis is felt to be an important initiator of leukocyte/keratinocyte interactions in many inflammatory skin diseases. The purpose of this project was to determine the individual variability of cytokine-induced ICAM-1 expression in human keratinocytes obtained from different donors. In 55 different keratinocyte strains, there was significant individual variability in ICAM-1 expression by either tumor necrosis factor α (TNF-α) or interferon-γ. There was no correlation ($r = 0.266$, $p = 0.06$) in response of the same strain to either TNF-α or interferon-γ. Multiple ($n = 22$) keratinocyte strains showed no significant induction of ICAM-1 expression to IL-1. The level of ICAM-1 expression in response to TNF-α and ultraviolet radiation (UVR) in individual strains was highly correlated in three different comparisons: level of stimulated response versus baseline (TNF-α and UVR both $p < 0.0001$); stimulation index TNF-α versus UVR ($p = 0.00947$); and variability of stimulated response versus variability of baseline (TNF $p < 0.001$; UVR $p = 0.002$). UVR-induced release of TNF from keratinocytes also showed variability among different keratinocyte strains. The UVR-induced ICAM-1 response in human keratinocytes and transformed epithelial cell was variably blocked with anti-TNF antibodies. The release of TNF from keratinocytes by UVR and the individually variable but linked characteristics of UVR and TNF-α stimulated ICAM-1 expression support the hypothesis that TNF-α is a major mediator of UVR-induced ICAM-1 expression. *J Invest Dermatol 104:489–496, 1995*

Intercellular adhesion molecule-1 (ICAM-1, CD54) is the prototype of adhesion molecules that initiate cellular interactions in the immune response. ICAM-1 is a ligand for the B-2 integrin leukocyte function–associated antigen-1 (LFA-1) [1], found on most leukocytes. The ICAM-1/LFA-1 interaction is necessary to initiate many of the important immunologic functions that require leukocyte/leukocyte, leukocyte/endothelial cell, or leukocyte target interaction [2–4]: specific antigen recognition by B and T lymphocytes [4–6], superantigen stimulation of T lymphocytes [7], leukocyte migration into tissue [8], and leukocyte-mediated cytotoxicity [9,10]. In keratinocytes there is very low constitutive expression of cell surface ICAM-1 [11], in contrast to endothelial cells, fibroblasts, and inflammatory cells. Interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α) and -β and ultraviolet radiation (UVR), but not IL-1, induce increased ICAM-1 expression in keratinocytes [11–13]. It has been proposed that such differences in ICAM-1 expression are important in the functional differences among skin cells during immune responses [9,14].

To test whether there are individual differences in the patterns of ICAM-1 induction by different cytokines, we tested the individual variability of inductions of ICAM-1 expression in 55 keratinocyte strains. Correlations in the responses were analyzed, and possible connections between the response to UVB and TNF-α were further investigated.

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Reprint requests to: Dr. David A. Norris, Department of Dermatology, B-144, University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, CO 80262.

MATERIALS AND METHODS

Keratinocyte Culture Human neonatal foreskin keratinocytes were cultured as previously described [13] in keratinocyte growth medium (Gibco, Grand Island Biological Company, Grand Island, NE). Cell cultures were used at the second or third passage and studied at 50–75% confluence.

Foreskins were obtained from an adjacent hospital after a short period in refrigeration. Individual keratinocyte strains were produced from single foreskins whenever possible, although a few strains were produced from two or three foreskins when the tissue samples were small.

In experiments using adult donors, epidermal suction blisters were obtained from volar forearm skin.

TNFα levels Keratinocytes of a single strain were cultured in six-well culture plates (Falcon) for 24 h. Fresh media was added with the indicated level of calcium, and the cells were cultured for a further 24 h. Before irradiation, culture media was removed and a minimal amount of phosphate-buffered saline (PBS) added to keep cells moist. After irradiation, culture media was replaced, maintaining appropriate calcium levels. Cells were cultured after irradiation 3 d and cell culture media was removed for assay. TNFα concentrations were determined by a commercial enzyme-linked immunosorbent assay kit (Genzyme, Cambridge, MA).

Cell cultures were radiated with three light sources. UVB radiation was delivered by a Dermalight 2001 Sol 3 Solar Simulator (Dermalight Systems, Studio City, CA), or by FS 20 bulbs (National Sun Lamp). UVA was delivered by a Houva F24.12BL lamp. Radiance of UVA or UVB was

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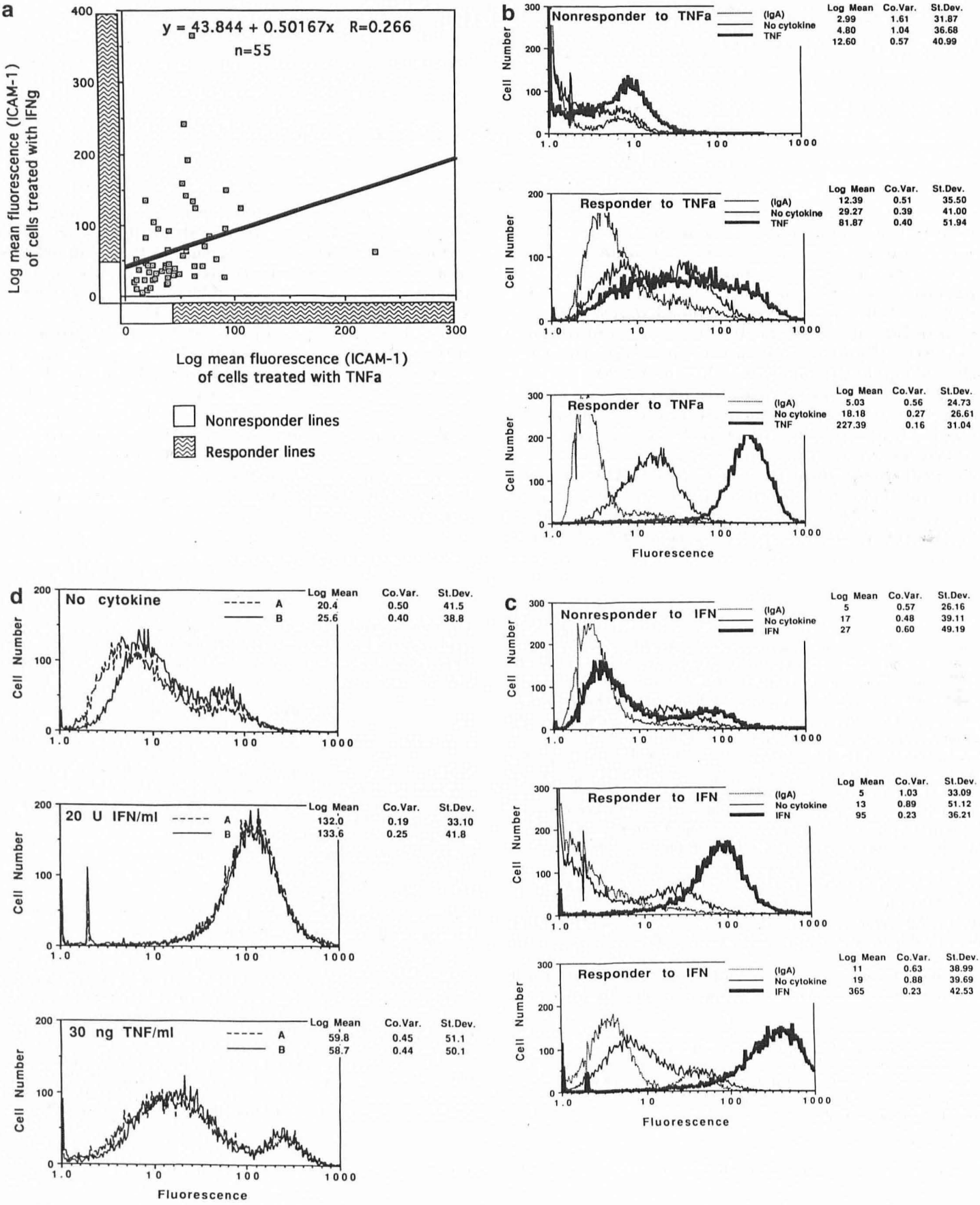
a
y = 43.844 + 0.50167x R=0.266
n=55
Log mean fluorescence (ICAM-1) of cells treated with IFNg
Log mean fluorescence (ICAM-1) of cells treated with TNFa
Nonresponder lines
Responder lines
b
Nonresponder to TNFa
Responder to TNFa
Responder to TNFa
(IgA)
No cytokine
TNF
Log Mean Co.Var. St.Dev.
2.99 1.61 31.87
4.80 1.04 36.68
12.60 0.57 40.99
12.39 0.51 35.50
29.27 0.39 41.00
81.87 0.40 51.94
5.03 0.56 24.73
18.18 0.27 26.61
227.39 0.16 31.04
Cell Number
Fluorescence
c
Nonresponder to IFN
Responder to IFN
Responder to IFN
(IgA)
No cytokine
IFN
5 0.57 26.16
17 0.48 39.11
27 0.60 49.19
5 1.03 33.09
13 0.89 51.12
95 0.23 36.21
11 0.63 38.99
19 0.88 39.69
365 0.23 42.53
d
No cytokine
20 U IFN/ml
30 ng TNF/ml
A
B
20.4 0.50 41.5
25.6 0.40 38.8
132.0 0.19 33.10
133.6 0.25 41.8
59.8 0.45 51.1
58.7 0.44 50.1

measured by an IL radiometer IL1700/760D/791 (International Light Radiometer, Newbury, MA). All cultures were irradiated in PBS with the culture dish uncovered at a distance of 6.5 cm from the light source.

ICAM-1 Expression Cell surface ICAM-1 was measured by flow-cytometry analysis as described [13]. Cultured keratinocytes were grown in six-well plates for 1 to 2 d before the addition of cytokine. After the appropriate incubation period, usually 3 d for TNF-α and 1 d for IFN-γ, the keratinocytes were harvested by brief trypsinization. The cells were then stained with anti–ICAM-1 (kindly supplied by Robert Rothlein, Boehringer-Ingelheim), followed by fluorescein isothiocyanate–goat anti-mouse IgG (Fisher). Cells reacted with mouse anti-human IgA acted as an isotype control. Flourescence was analyzed on an EPIC fluorescence-activated cell sorter (FACS). Results are expressed as log mean fluorescence. Delta log mean indicates non-specific fluorescence (negative control) has been subtracted.

Cytokines Human recombinant TNF-α and IFN-γ from Genzyme were used throughout.

Blocking Experiments Two different anti–TNF-α antibodies were used. Polyclonal rabbit anti-human TNF-α was obtained as whole serum from Genzyme. Monoclonal mouse anti-human TNF-α and anti–interleukin-1α were obtained from Upstate Biotechnology Inc. (New York). To block exogenous cytokine, the antibody (0.5 μg/ml) and cytokine were mixed in culture media at room temperature for 30 min before adding to cell culture. To block UV-induced cytokine, the antibody (0.5 μg/ml) was added to cell culture media immediately following UV irradiation. The keratinocytes were incubated for the usual length of time before cell surface ICAM-1 was assayed.

RESULTS

Responders Versus Nonresponders to TNF-α and IFN-γ In examination of 55 different keratinocyte strains obtained from single donors or from multiple donors, we found that there was great variability in the level of response and in the pattern of response. We arbitrarily divided keratinocyte strains into responders (log mean 50 or greater) and nonresponders (log mean less than 50) after stimulation. Each of 55 strains was stimulated with TNF-α (30 ng/ml) or IFN-γ (20 U/ml), and the expression of ICAM-1 measured by FACS. There is no correlation between the response to IFN and that to TNF-α **(Fig 1*a*)** by regression analysis ($r = 0.266$, $p = 0.06$). Some strains are responders to TNF-α but nonresponders to IFN-γ, and vice versa.

The FACS profiles from a nonresponder and two responder strains are shown in **Fig 1*b*** for TNF-α and **Fig 1*c*** for IFN-γ. Responders show either broad or narrow peaks shifted to the right, whereas nonresponders show limited shifts in response to cytokines.

Responder status is a reproducible characteristic. In one neonatal foreskin keratinocyte strain that is both a TNF and IFN-γ responder, the level of response in experiments performed at two different times is nearly identical **(Fig 1*d*)**. Numerous experiments using keratinocytes from the same adult donor harvested at different times by suction blister from forearm skin have shown that responder status is a reproducible characteristic (data not shown).

Lack of Variability in Interleukin 1 (IL-1) Induction of ICAM-1 Expression No significant induction of ICAM-1 expression was seen in keratinocytes stimulated with IL-1. As shown in **Fig 2**, IL-1α produced no significant enhancement in baseline expression of ICAM-1 in 22 keratinocyte strains. If this data is expressed as stimulation index **(Fig 2*B*)**, only three of 20 strains showed a stimulation index greater than 1.5, and these relative increases were only seen at very low baseline ICAM-1 levels. This is in no way comparable to the exuberant ICAM-1 induction with TNF-α or IFN-γ.

UVR-Induced Release of TNF-α from Keratinocytes UVB-dominant light sources produce a dose-dependent release of TNF-α in keratinocyte cultures, as shown in **Fig 3**. This release is greatest in more differentiated cells in high-calcium medium. Baseline release from the cultures is not appreciably different in the three medium conditions tested. The increased TNFα release in more differentiated cells is seen in spite of the fact that the total number of cells decreases in these less proliferative cultures. UVA sources do not induce TNF-α release. As shown in **Table I**, the levels of TNF-α release from four different keratinocyte strains showed considerable individual variability. No significant TNF-α release was seen with control keratinocytes.

Relationships Among the Responses to IFN-γ and TNF-α and UVR We next considered whether keratinocyte strains that responded to TNF-α also responded to UVR. With all three stimuli (TNF-α, UVR, or IFN-γ) the level of ICAM-1 induction was directly related to the baseline ICAM-1 expression: IFN-γ, $p = 0.009$ **(Fig 4*A*)**, TNF-α, $p = 0.0001$ **(4*B*)** and UVR, $p = 0.0001$ **(4*C*)**. To correct for this strong correlation, the value for the stimulated ICAM-1 expression was divided by the level of baseline ICAM-1, producing a value called stimulation index. In comparing the stimulation indices produced by IFN-γ, TNF-α, and UVR **(Fig 5)**, there is a strong correlation between the responses to TNF-α and UVR ($r = 0.591$, $p = 0.0047$).

The relationship between response to TNF-α and UVR is also seen in the variability of ICAM-1 response as measured by correlation of variability with both TNF-α and UVR, there was a strong correlation between the correlation of variability at baseline and the coefficient of variation after stimulation (data not shown). This was not seen in IFN-γ–stimulated cultures.

The correlations of responsers of individual strains to TNF-α and UVR are summarized in **Table II**. Careful analysis of other relationships among the data show no further correlations (data not shown).

Blocking of UVR-Induced ICAM-1 Expression with Anti–TNF-α Antibodies to TNF-α produced variable inhibition of the UVR-induced ICAM-1 expression in keratinocytes strains and in transformed keratinocyte cell lines. As shown in **Table III**, addition of exogenous rabbit polyclonal anti–TNF-α produced up to 70% inhibition of UVR-induced ICAM-1, but did not influence the high ICAM-1 expression induced by IFN-γ. However, this rabbit antiserum itself produced some toxicity of keratinocytes as evidenced by morphologic change and detachment of some cells. It did not, however, so strongly affect the responses of adherent keratinocytes as to block the strong induction of ICAM-1 expression by IFN-γ **(Table III)**.

Additional experiments were performed with mouse monoclonal anti–TNF-α and anti–IL-1. **Figure 6** shows ICAM-1 expression in cultured human keratinocytes following stimulation with IFN, TNF, TNF plus IFN, UVR, and UVR plus IFN, and the effects of addition of anti–IL-1 or anti–TNF-α. Although anti–TNF-α would

Figure 1. Different keratinocyte strains can be classified as nonresponders or responders based on induction of ICAM-1 expression by TNF or IFN. *a*: Cytokine-induced ICAM-1 expression (log mean fluorescence) in 55 different keratinocyte strains is shown in paired cultures treated either with TNF-α 30 ng/ml for 3 d or IFN-γ 20 U/ml for 1 d (times of optimal response). The keratinocyte strains are arbitrarily divided into nonresponders (log mean ICAM-1 less than 50) or responders (greater than 50) based on the cell surface ICAM-1 measured by FACS (log mean). Regression analysis of the data shows that in the same strains of cells there is no relationship between ICAM-1 induction by TNF-α versus IFN-γ ($r = 0.266$, $p = 0.06$). *b*: Cell surface ICAM-1 expression in cultured human keratinocyte following TNF-α: identification of responder and nonresponder strains. FACS profiles and quantitative analysis of ICAM-1 (log mean) and variability in ICAM-1 (Co. Var. and St. Dev.) is shown for three different keratinocyte strains: one nonresponder to TNF and two responders to TNF. Three FACS profiles are shown in each panel: isotope-matched negative control (IgA), baseline (no cytokine), and TNF-α stimulated (TNF). *c*: Cell surface ICAM-1 expression in cultured human keratinocytes following IFN: identification of responder and nonresponder strains. Similar organization to *b*. *d*: Reproducibility in ICAM-1 expression induced by IFN-γ or TNF-α. Aliquots of the same keratinocyte strain were thawed and tested on two subsequent weeks, noted as dotted (A) and solid (B) lines. The profiles and quantitative data were quite similar.

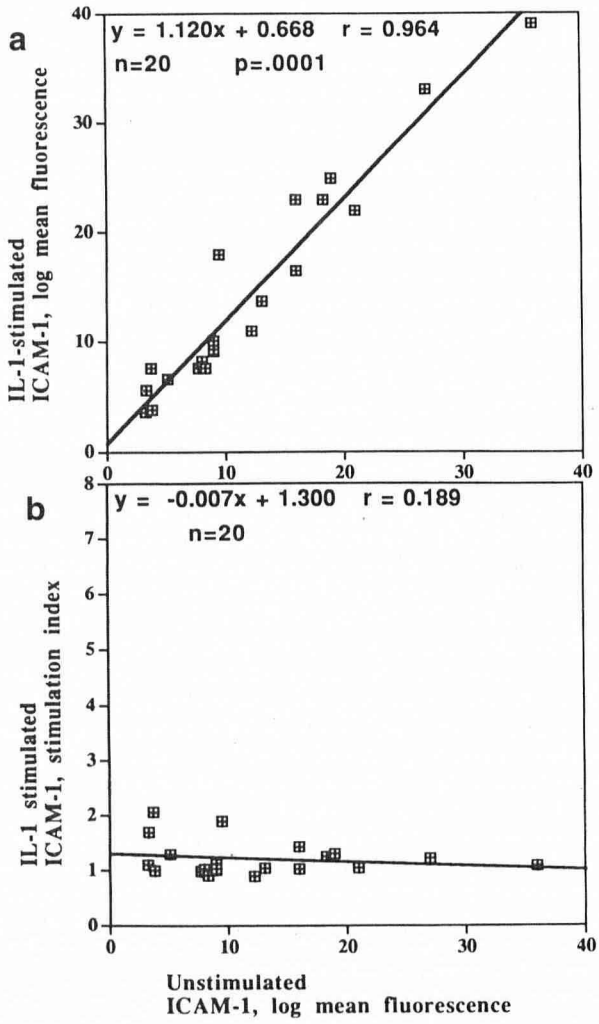

Figure 2. Effects of IL-1 on ICAM-1 expression in 22 different keratinocyte strains. *a*: Comparison of baseline ICAM-1 expression (no cytokine) versus expression after IL-1 stimulation. *b*: Comparison of baseline ICAM-1 expression versus the "stimulation index" (stimulated/baseline) after IL-1 stimulation.

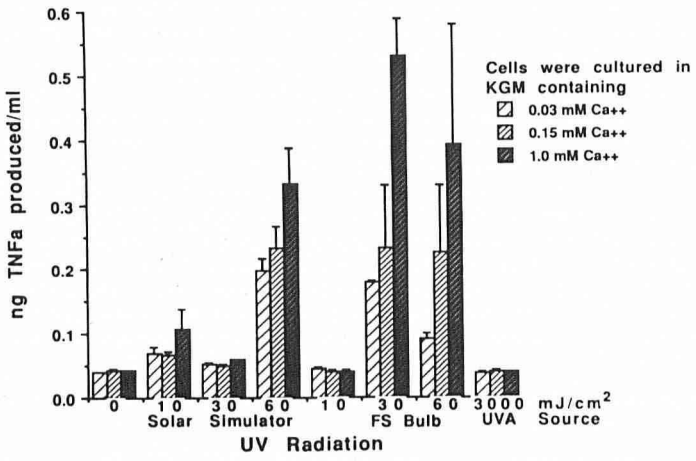

Figure 3. TNF-α production in cultured neonatal human keratinocytes in response to ultraviolet radiation. The *bars* represent the mean and the brackets the SEM of three replicates. Three levels of differentiation of cultured keratinocytes were induced by use of three different calcium concentrations (0.03, 0.15, 1.0 mM) in the keratinocyte growth medium media. Three radiation sources were used: solar simulator, FS bulb, and UVA source. The radiation dose (mJ/cm^2) noted for each source is the measured UVB dose in the solar simulator and FS source, and the UVA radiance in the UVA source. TNF-α in cell supernatants was measured by enzyme-linked immunosorbent assay.

Table I. TNF Release from Keratinocytes Following UV Radiation[a]

	Released TNFα (pg/ml)[b]	
Subject	Solar Simulator	FS Bulb
1	121 ± 25[c]	115 ± 11
2	334 ± 26	532 ± 58
3	60 ± 12	118 ± 2
4	Not done	770 ± 223

[a] Irradiation with 100 mJ/cm^2 from solar simulator.
[b] Control or sham-irradiated cultures showed released TNF-α below the level of detection of the assay.
[c] Mean ± SEM for triplicate measurements.

block induction of ICAM-1 expression by exogenous TNF-α, neither anti–IL-1 nor anti–TNF-α produced any significant inhibition of the synergistic effect of UVR with IFN. This particular keratinocyte strain was a non-responder to UVR alone.

Experiments with the keratinocyte cell line A431 and KB also failed to demonstrate the activity of mouse monoclonal anti-TNF or anti–IL-1 in blocking UVR-induced ICAM-1 expression. As shown in **Figure 7**, these antibodies significantly blocked the effect of exogenous IL-1 in stimulating ICAM-1 expression in KB cells, or the effect of anti–TNF-α in inducing ICAM-1 expression in A431 and KB cells. However, neither antibody significantly blocked the UVR-induced ICAM-1 expression in these two cell lines.

DISCUSSION

The major finding of this study is that individual keratinocyte strains show considerable variability in baseline ICAM-1 expression, and in the pattern of ICAM-1 expression induced by IFN-γ, TNF-α, and UVR. Responders and non-responders can be observed with IFN-γ, TNF-α, and UVR. IL-1 produced no significant ICAM-1 responses in the keratinocyte strains tested. The levels of ICAM-1 induced by TNF-α or by UVR were significantly correlated in the strains studied.

Cell strains were all studied in early passage to assure that the phenotypes were similar to cells *in vivo*. The variability observed among the different culture doses does not represent some random effect due to poor handling of the original foreskin samples, differences in confluence of culture, or other identifiable artifacts of preparation. All cultures were tested just prior to confluence at the shortest time possible in culture. The baseline and stimulated levels of ICAM-1 expression were reproducible characteristics of the strains tested.

The lack of association between IFN-γ and TNF-α responses indicates that these are independent variables, controlled by different receptors, and probably by different cell signaling pathways and nuclear response elements. This is supported by recent results that demonstrate a unique IFN-γ–specific responsive element of the ICAM-1 gene in epithelial cells,* and unique activation of the TNF-α–responsive element of the ICAM-1 gene by p65 NFkB.†

The low constitutive expression of ICAM-1 in keratinocytes [11,15] is determined by a promoter region that suppresses ICAM-1 gene transcription [16]. Induction of ICAM-1 expression in kera-

* Naik S, Shibagaki N, Li J-L, Caughman SW: IFN-g activates a novel trans-acting factor in epithelial cells that binds to the IFN-g response element (RE) of the human ICAM-1 gene (abstr). *J Invest Dermatol* 102:535, 1994.

† Li L-J, Naik S, Shibagaki N, Caughman SW: Identification of the tumor necrosis factor (TNF) responsive region of the human ICAM-1 gene (abstr). *J Invest Dermatol* 102:557, 1994.

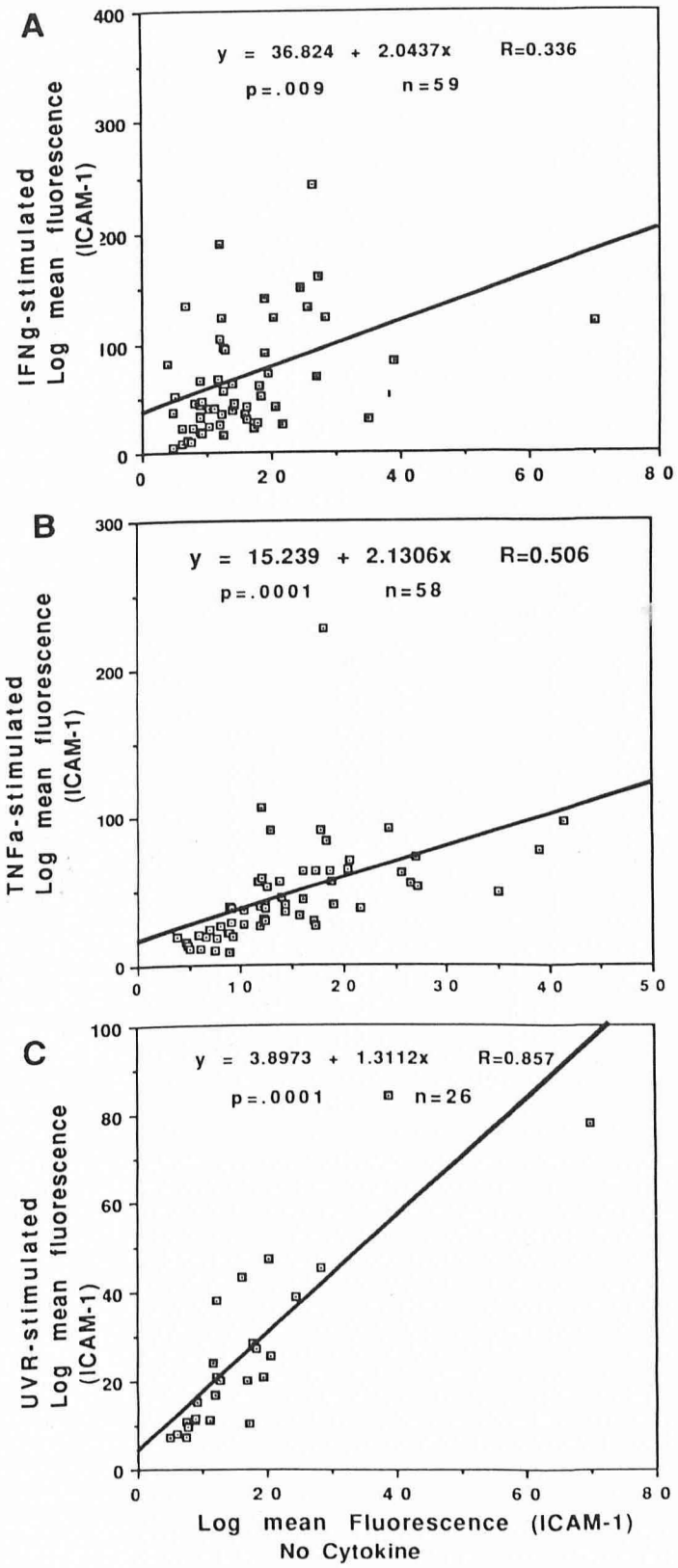

Figure 4. Comparison of cell surface ICAM-1 expression (log mean fluorescence) on human keratinocytes without stimulation (abscissa, no cytokine) and with stimulation (ordinate). In *A*, *B*, and *C*, the cells were stimulated with IFN, TNF, or UVR, respectively. Individual points represent results with individual keratinocyte strains. All of the strains shown in *C* were also included in *A* and *B*; all of the strains shown in *B* are also shown in *A*. The degree of response to all three stimuli is significantly related to the level of baseline ICAM-1 expression: *A*, r = 0.336, p = 0.009; *B*, r = 0.506, p = 0.001; *C*, r = 0.857, p = 0.001.

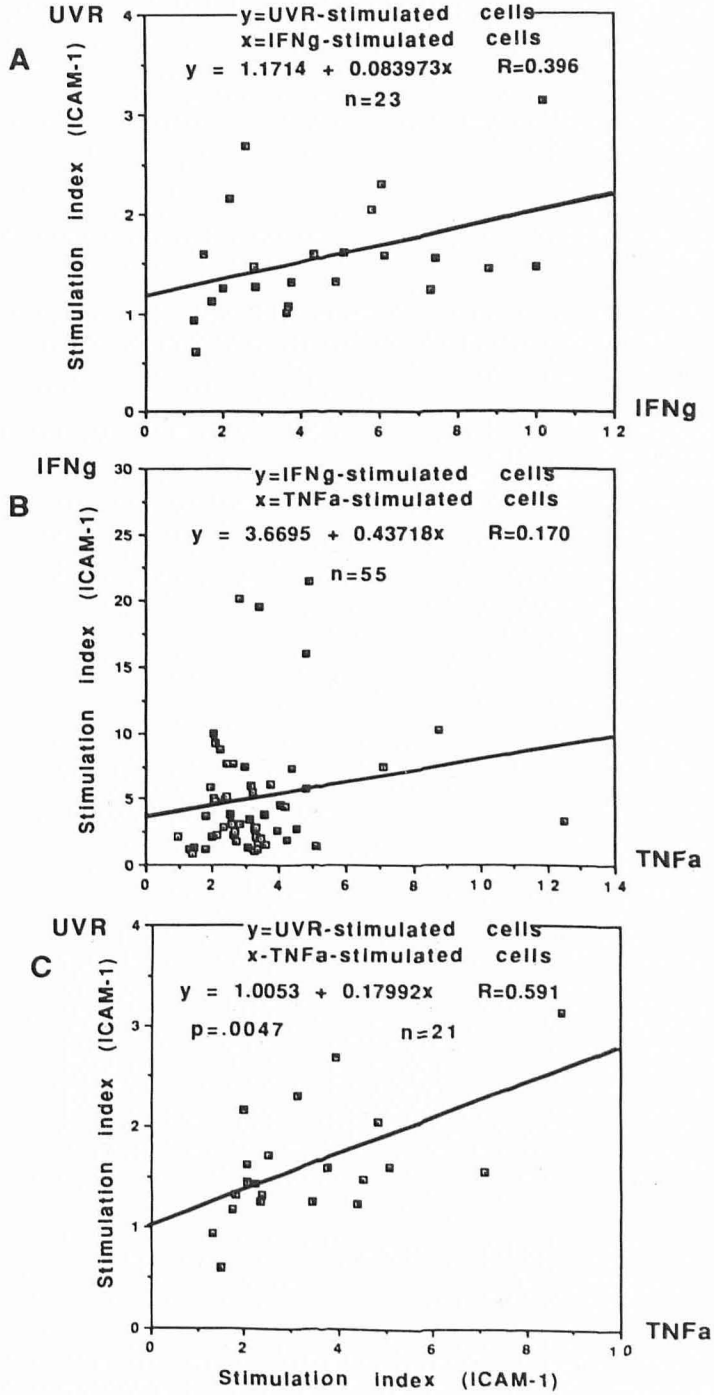

Figure 5. Study of the relationship between induction of ICAM-1 expression by TNF, IFN, or UVR. ICAM-1 response to the three stimuli are adjusted for baseline ICAM-1 expression and are expressed as stimulation indices (stimulated/baseline). Regression analysis is used to analyze the relationship between response to the three stimuli: *A*, UVR versus IFN-γ; *B*, IFN-γ versus TNF-α; *C*, UVR versus TNF-α. In each graph, the individual points represent the response of separate keratinocyte strains to the two stimuli, and are shown with regression lines, Y intercept, and R and p values. No significant correlations were seen in two of the paired responses: TNF-α/IFN-γ and UVR/IFN-γ. However, there was significant correlation in the level of response of 21 keratinocyte strains to TNF/UVR (r = 0.591, p = 0.0047).

tinocytes is highly regulated in keratinocytes through activation of IFN-γ receptors [17], IL-1 type I receptors [18], and the 55-kD TNF-α receptors [19]. In keratinocytes, activation of the IL-1 receptor may be inhibited by the IL-1 receptor antagonist (IL-1ra), which is found in keratinocytes IL-1ra as an intracellular molecule

Table II. Effects of Anti-TNF-α on ICAM-1 Expression Induced by UVR

	IFN-g	UVR
Increase in ICAM-1 expression over control	52	40
Blocking by anti-TNF[a]		
10 μ/ml	0%	10%
100 μ/ml	0%	75%

[a] Percent inhibition by preincubation with anti-TNF-a rabbit serum diluted 1:30 in culture medium.

Table III. Correlations in Induced ICAM-1 Expression of Individual Keratinocyte Strains to TNF-α and UVR

	IFN-γ	TNF	UVR
Correlation of induced ICAM-1 to baseline ICAM-1 expression **(Fig 5)**	p = 0.009	p = 0.0001	p = 0.0001
Stimulation index of ICAM-1 **(Fig 6)**	None	TNF and UVR correlate by regression analysis p = 0.0047	
Correlation of variability in stimulated response baseline variability	None	p = 0.0001	p = 0.0002

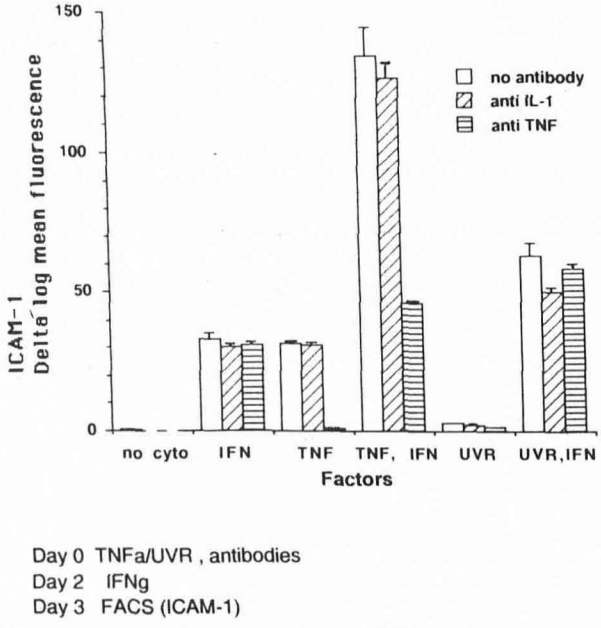

Figure 6. Effect of blocking antibodies on induction of ICAM-1 expression in cultured human keratinocytes by treatment with IFN, TNF, UVR, or combinations of these. The *bars* and *brackets* represent mean ± SEM of four points. Function blocking antibodies to TNF (anti-TNF 0.5 μg/ml) or IL-1 (anti-IL-1 0.5 up/ml) were added to cultured human keratinocytes at day zero when the cells were stimulated with TNF-α or UVR. In some conditions, IFN was added to cells at day 2. ICAM-1 expression was measured at day 3 by FACS analysis. In some conditions, cells were stimulated with either TNF at day 0, then with IFN at day 2 to study synergy (TNF, IFN) or with UVR at day 3 and IFN-β at day 2 (UVR, IFN). Significant inhibition of responses was seen with anti-TNF in TNF-α–stimulated cells, but neither anti-TNF nor anti-IL-1 affected UVR-induced responses.

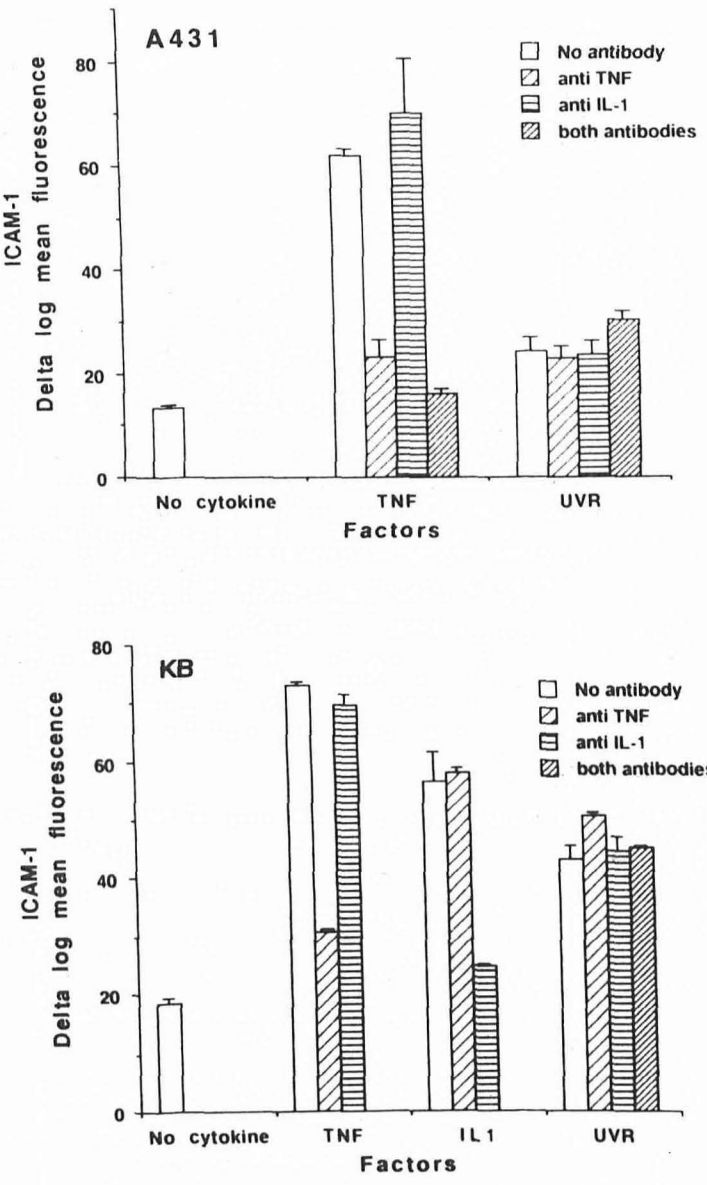

Figure 7. The effect of function-blocking antibodies to IL-1 (anti-1) or TNF-α (anti-TNF) on ICAM-1 induction by TNF-α or UVR in A431-transformed keratinocytes or by TNF-α, IL-1 or UVR in KB-transformed keratinocytes. The *bars* and *brackets* represent the mean ± SEM of four replicate points. TNF-α, IL-1, or UVR were applied at day 0 along with control or antibodies, and ICAM-1 expression was measured at day 3. With A431 cells, significant blocking of TNF-α induced ICAM-1 expression was seen with anti-TNF (p = 0.01) or with both antibodies (p = 0.002). With UVR-induced ICAM-1 expression, neither antibody blocked induction. In KB cells, significant blocking of TNF-α–induced ICAM-1 expression was seen with anti-TNF (p = 0.01) and of IL-1–induced ICAM-1 by anti–IL-1 (p = 0.01). However, neither antibody blocked UVR-induced ICAM-1 expression.

in twentyfold excess over IL-1a [20]. TNF-α or -β binding to the 55-kD receptor can be blocked by the soluble TNF-INH that is a proteolytic fragment of the receptor [21]. IFN-γ and TNF synergistically activate transcription of the ICAM-1 gene [22]; both IFN- and TNF-responsive elements are present in the promoter region of the ICAM-1 gene [16,23]. An apparent IL-1–responsive element is also present, but is not inducible when the gene is transfected into keratinocytes [16]. Activation of the ICAM-1 gene by IL-1 may be selectively inhibited by the large excess levels of the intracellular form of IL-1ra found in epidermal keratinocytes.‡

Ultraviolet light has a biphasic effect on ICAM-1 expression, inhibiting induction of ICAM-1 expression in the first 24 h and then

‡ Middleton M, Arend W, Norris D: High intracellular interleukin-1 receptor antagonist (icIL-1ra) levels are correlated with low expression of intracellular adhesion molecule-1 in keratinocytes and fibroblasts (abstr). *J Invest Dermatol* 102:524, 1994.

enhancing ICAM-1 expression [12,13]. In the first 24 h following UVR, ICAM-1 induction is inhibited by interference with transcription through disruption of transcription factors [24]. The late effects of UVR on ICAM-1 appear to be mediated through TNF-α release. In some keratinocyte lines, UVR-released TNF upregulates the 55-kD TNFR mRNA in an autocrine fashion [25]. In our own experiments, priming with TNF enhances subsequent induction of ICAM-1 expression by TNF-α or UVR.§

UVR induces TNF-α release from keratinocytes [26]. We show that this release is UVB (but not UVA) dependent, and is greatest in more differentiated keratinocytes. There is strong circumstantial evidence that the release of TNF-α by UVR is dependent on the isomerization of trans to cis UCA [27]; trans-UCA is most abundant in more differentiated keratinocytes. Release of TNF-α also showed considerable variability in keratinocytes from different individuals.

IL-1 is also an important cytokine that may be induced by UVR. UVB radiation induces transcription of the gene for IL-1 [28], and induces circulating levels of IL-1–like activity in irradiated humans [29]. However, keratinocyte IL-1 is an intracellular molecule without a signal sequence necessary for secretion. IL-1a is released from monocytes and macrophages by induction of cell damage [30] or death by necrosis or apoptosis [31]. Both IL-1 and IL-1ra probably require significant keratinocyte damage to induce release.

The reasons for the differences in the blocking patterns of polyclonal and monoclonal antibodies to TNF-α in blocking UVR-induced ICAM-1 expression are not clear. It is possible that TNF-α acts in an autocrine or juxtacrine fashion not always influenced by extracellular antibodies. Functional cell-bound TNF has been described in some systems [32,33], and may be variably blocked by antibodies that usually block soluble cytokine function.

Grewe *et al* [34] and colleagues have shown that UVR-induced ICAM-1 expression on transformed epithelial cells (KB cells), and found that anti–IL-1 and anti–TNF-α both blocked UVR-induced prostanoid release. They have proposed that UVR may release IL-1 and TNF and that both might up-regulate TNFR expression [25,34]. In our hands, there are considerable differences between the behavior of different keratinocyte-transformed cell lines and cultured human keratinocytes. Short-term cultures of human keratinocytes and transformed cell lines such as A431 and HaCaT have high constitutive levels of IL-1ra, associated with low-baseline ICAM-1 expression and low induction of ICAM-1 expression by IL-1. The KB cell line, on the other hand, has low IL-1ra levels, high-baseline ICAM-1 expression, and strong induction by TNF-α and IL-1.‡ One must be cautious in extrapolating IL-1–dependent effects from KB cells to keratinocytes.

There is now evidence that the individual differences observed in these experiments are genetically based. Foreskin cultures are not amenable to longitudinal study. In subsequent experiments,§ we have found that keratinocyte cultures derived from repeated biopsy of adults show reproducible ICAM-1 responses to TNF-α *in vitro*, and that those individuals who are responders to TNF-α *in vitro* are also responders to UVR *in vivo*.

We have found that baseline ICAM-1 expression correlates with the levels of icIL-1ra, both in transformed keratinocyte cell lines and in fibroblasts transfected with the cDNA for icIL-1ra.‡ This raises the possibility that polymorphisms in icIL-1ra may determine baseline ICAM-1 levels, which would determine the level of subsequent TNF response. A strong relationship between baseline ICAM-1 and TNF-α–stimulated ICAM-1 expression is shown in the results reported here **(Fig 4)**. Future studies on the association of effects of TNF-α and IL-1ra polymorphisms may clarify this issue.

Recently, the importance of genetic polymorphism in both TNF and IL-1ra genes have been appreciated. High TNF-α response is linked to the DR3 gene [35], associated with autoantibody responses in a number of diseases including those of many patients with photosensitive lupus [36,37]. One IL-1ra polymorphism is strongly correlated with photosensitive chronic cutaneous lupus erythematosus.¶ Particular polymorphisms in TNF-α or IL-1ra may determine keratinocyte ICAM-1 expression and influence disease development in cutaneous lupus or other skin diseases.

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REFERENCES

1. Marlin SD, Springer TA: Purified intercellular adhesion molecule (ICAM-1) is a ligand for lymphocyte function associated antigen. *Cell* 51:813–819, 1987
2. Boyd AW, Wawryk SO, Burns GF, Fecondo JV: Intercellular adhesion molecule 1 (ICAM-1) has a central role in cell-cell contact dependent mediated immune mechanism. *Proc Natl Acad Sci USA* 85:3095–3099, 1988
3. Lanier LL, Ruitenberg JJ, Phillips JH: Functional and biochemical analysis of CD16 antigen on natural killer cells and granulocytes. *J Immunol* 141:3478–3485, 1988
4. Makgoba MW, Sanders ME, Ginther Luce GE, Dustin ML, Springer TA, Clark EA, Mannori P, Shaw S: ICAM-1 a ligand for LFA-1-dependent adhesion by B, T and myeloid cells. *Nature* 331:86–91, 1988
5. Damle NK, Klussman K, Linsley PS, Aruffo A: Differential costimulatory effects of adhesion molecules B7, ICAM-1, LFA-3, and VCAM-1 on resting and antigen-primed CD4+ T lymphocytes. *J Immunol* 148:1985–1992, 1992
6. Lane PJ, McConnell FM, Clark EA, Mellins E: Rapid signaling to B cells by antigen-specific T cells requires CD18/CD54 interaction. *J Immunol* 147:4103–4108, 1991
7. Fischer H, Gjorloff A, Hedlund G, Hedman H, Lundgren E, Kalland T, Sjogren HO, Dohlsten M: Stimulation of human naive and memory T helper cells with bacterial superantigen. Naive CD4+45RA+ T cells require a costimulatory signal mediated through the LFA-1/ICAM-1 pathway. *J Immunol* 148:1993–1998, 1992
8. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC: Cooperative interactions of LFA-1 and MAC-1 with intercellular adhesion molecule 1 in facilitating adherence and trans endothelial migration of human neutrophils in vitro. *J Clin Invest* 83:2008–2017, 1989
9. Norris DA: Cytokine modulation of adhesion molecules in the regulation of immunologic cytotoxicity of epidermal targets. *J Invest Dermatol* 95:111S–120S, 1990
10. Makgoba MW, Sanders ME, Ginther Luce GE, Gugel EA, Dustin ML, Springer TA, Shaw S: Functional evidence that intercellular adhesion molecule 1 (ICAM-1) is a ligand for LFA-1 dependent adhesion in T cell-mediated cytotoxicity. *Eur J Immunol* 18:637–640, 1988
11. Dustin ML, Singer KH, Tuck DT, Springer TA: Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon-gamma and is mediated by intercellular adhesion molecule-1 (ICAM-1). *J Exp Med* 167:1323–1340, 1988
12. Krutmann J, Kock A, Schauer E, Parlo F, Moller A, Kapp A, Forster E, Schopf E, Luger TA: Tumor necrosis factor beta and ultraviolet radiation are potent regulators of human keratinocyte ICAM-1 expression. *J Invest Dermatol* 95:127–131, 1990
13. Norris DA, Lyons MB, Middleton MH, Yohn JJ, Kashihara Sawami M: Ultraviolet radiation can either suppress or induce expression of intercellular adhesion molecule 1 (ICAM-1) on the surface of cultured human keratinocytes. *J Invest Dermatol* 95:132–138, 1990
14. Norris DA: Immunological cytotoxicity of cutaneous cellular targets. In: Norris DA (ed.). *Immune Mechanisms in Cutaneous Disease*. Marcel Dekker, Inc., New York, 1989, pp 123–166
15. Yohn JJ, Critelli M, Lyons MB, Norris DA: Modulation of melanocyte intercellular adhesion molecule-1 by immune cytokines. *J Invest Dermatol* 95:233–237, 1990
16. Cornelius LA, Taylor JT, Degitz K, Li LJ, Lawley TJ, Caughman SW: A 5′ portion of the ICAM-1 gene confers tissue-specific differential expression levels and cytokine responsiveness. *J Invest Dermatol* 100:753–758, 1993
17. Mitra RS, Nickoloff BJ: Epidermal growth factor and transforming growth factor-alpha decrease gamma interferon receptors and induction of intercellular adhesion molecule (ICAM-1) on cultured keratinocytes. *J Cell Physiol* 150:264–268, 1992
18. Kupper TS, Lee F, Birchall N, Clark S, Dower S: Interleukin 1 binds to specific receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. A potential autocrine role for interleukin 1 in epidermis. *J Clin Invest* 82:1787–1792, 1988
19. Trefzer U, Brockhaus M, Loetscher M, Parlow F, Kapp A, Schopf E, Krutmann J: 55-kd tumor necrosis factor receptor is expressed by human keratinocytes and plays a pivotal role in regulation of human keratinocyte ICAM-1 expression. *J Invest Dermatol* 97:911–916, 1991

§ Bennion SD, Middleton MH, David Bajar KM, Brice S, Norris DA: Determinants of different patterns of expression of keratinocyte intercellular adhesion molecule-1 (ICAM-1) in skin diseases (manuscript submitted).

¶ Cork MJ, Tarlow JK, Blakemore AJF, McDonagh AJG, Messenger AG, Bleehan SS, Duff GW: Genetics of Interleukin I receptor antagonist in inflammatory skin diseases (abstr). *J Invest Dermatol* 100:522, 1993.

20. Bigler CF, Norris DA, Weston WL, Arend WP: Interleukin-1 receptor antagonist production by human keratinocytes. *J Invest Dermatol* 98:38–44, 1992
21. Seckinger P, Zhang JH, Hauptmann B, Dayer JM: Characterization of a tumor necrosis factor alpha (TNF-alpha) inhibitor: evidence of immunological cross-reactivity with the TNF receptor. *Proc Natl Acad Sci USA* 87:5188–5192, 1990
22. Barker JNWN, Sarma V, Dixit V, Mitra RS, Nickoloff BJ: Marked synergism between tumor necrosis factor alpha and interferon-gamma in regulation of keratinocyte-derived chemotactic factors and adhesion molecules. *J Clin Invest* 95:605–608, 1990
23. Caughman SW, Li LJ, Degitz K: Human intercellular adhesion molecule-1 gene and its expression in the skin. *J Invest Dermatol* 98:61S–65S, 1992
24. Krutmann J, Czech W, Parlow F, Trefzer U, Kapp A, Schopf E, Luger TA: Ultraviolet radiation effects on human keratinocyte ICAM-1 expression: UV-induced inhibition of cytokine-induced ICAM-1 mRNA expression is transient, differentially restored for IFN gamma versus TNF alpha, and followed by ICAM-1 induction via a TNF alpha-like pathway. *J Invest Dermatol* 98:923–928, 1992
25. Trefzer U, Brockhaus M, Lotscher H, Parlow F, Budnik A, Grewe M, Christoph H, Kapp A, Schopf E, Luger TA, *et al*: The 55-kD tumor necrosis factor receptor on human keratinocytes is regulated by tumor necrosis factor-alpha and by ultraviolet B radiation. *J Clin Invest* 92:462–470, 1993
26. Kock A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel JC, Luger TA: Human keratinocytes are a source for tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. *J Exp Med* 172:1609–1614, 1990
27. Kurimoto I, Streilein JW: Cis-urocanic acid suppression of contact hypersensitivity induction is mediated via tumor necrosis factor-alpha. *J Immunol* 148: 3072–3078, 1992
28. Kupper TS, Chua AO, Flood P, McGuire J, Gubler U: Interleukin 1 gene expression in cultured human keratinocytes is augmented by ultraviolet irradiation. *J Clin Invest* 80:430–436, 1987
29. Granstein RD, Sauder DN: Whole-body exposure to ultraviolet radiation results in increased serum interleukin-1 activity in humans. *Lymphokine Res* 6:187–193, 1987
30. Hogquist KA, Unanue ER, Chaplin DD: Release of IL-1 from mononuclear phagocytes. *J Immunol* 147:2181–2186, 1991
31. Hogquist KA, Nett MA, Unanue ER, Chaplin DD: Interleukin 1 is processed and released during apoptosis. *Proc Natl Acad Sci USA* 88:8485–8489, 1991
32. Perez C, Albert I, DeFay K, Zachariades N, Gooding L, Kriegler M: A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. *Cell* 63:251–258, 1990
33. Kriegler M, Perez C, DeFay K, Albert I, Lu SD: A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* 53:45–53, 1988
34. Grewe M, Trefzer U, Ballhorn A, Gyufko K, Henninger H, Krutmann J: Analysis of the mechanism of ultraviolet (UV) B radiation–induced prostaglandin E2 synthesis by human epidermoid carcinoma cells. *J Invest Dermatol* 101:528–531, 1993
35. Wilson AG, deVries N, Pociot F, diGiovine FS, vanderPutte LB, Duff GW: An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. *J Exp Med* 177:557–560, 1993
36. Sontheimer D, Maddison PJ, Reichlin M, Jordon RE, Stasyny P, Gilliam JN: Serologic and HLA associations in subacute cutaneous lupus erythematosus: a clinical subset of lupus erythematosus. *Ann Int Med* 97:664–671, 1982
37. Lee LA, Bias WB, Arnett FC Jr, Huff JC, Norris DA, Harmon C, Provost TT, Weston WL: Immunogenetics of the neonatal lupus syndrome. *Ann Intern Med* 99:592–596, 1983