The Activated Keratinocyte: A Model for Inducible Cytokine Production by Non-Bone Marrow–Derived Cells in Cutaneous Inflammatory and Immune Responses

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Keratinocytes, fibroblasts, endothelial cells, and other sessile cells resident to human skin can be induced in vitro to synthesize and secrete cytokine molecules. Cytokines are small protein molecules produced upon injury or cellular activation which influence immune and inflammatory events; as such, they have been best understood previously as products of leukocytes. The appreciation that cultured non-bone marrow–derived cells from skin could produce cytokines capable of initiating an inflammatory response or facilitating an immune response has led to speculation that cells resident to skin may be less passive participants in such phenomena than previously thought. Using as a model the cultured keratinocyte, which produces both interleukin-1 alpha and beta and an interleukin-1 receptor, models of autocrine and paracrine activation of this cell have been constructed. Such "activated keratinocytes," or by analogy other activated resident skin cells, produce a spectrum of cytokines in vitro which could potentially influence leukocyte adhesion to endothelium, direction migration of leukocytes towards the activated cell (presumably an inflammatory nidus), and activation of leukocyte functions in situ. The putative role of regulation of cytokine and cytokine-receptor regulation in mediating the activation of such cells (and thus, presumably, local inflammation) is discussed. An important aspect of this hypothetical model is that in the absence of activation (which characterizes normal uninflamed skin), cytokine production and its consequences do not occur. The conclusion reached is that based on in vitro data it is plausible to guess that local inflammatory or immune responses can be both initiated and facilitated by locally produced cytokines. *J Invest Dermatol 94:146S–150S, 1990*

As the compendium of cytokines produced by the cultured keratinocyte grows, there is an increasing urgency to understand the regulation of the production of these factors and their in vivo relevance. The term "activated keratinocyte" was developed primarily to emphasize that, at least in vitro, regulation of cytokine production can be addressed in a rigorous fashion, and that keratinocytes, like other cells, require an inductive signal for cytokine production. Certain features of keratinocyte cytokine production, however, may distinguish it from other cytokine-producing cells. For example, the observation that normal epidermis contains prodigious amounts of IL-1 alpha [1–4], and that cultured keratinocytes appear to produce this cytokine in the absence of intentional stimulation [5,6], suggests that keratinocytes may normally produce IL-1-alpha in vivo. In addition, the keratinocyte can express significant numbers of IL-1 receptors in vitro and in vivo [7–10], and can respond to IL-1 by producing numerous "secondary" cytokines (reviewed in [11]); thus, the potential for autocrine activation also distinguishes the keratinocyte from certain other cytokine-producing cells.

It is an axiom of cytokine biology that immune and inflammatory cells must be induced or "activated" to produce cytokines. "Resting" immune or inflammatory cells neither proliferate nor produce cytokines. For the resting T cell, the activating stimulus is antigen in the context of the appropriate MHC molecule. In contrast, the resting monocyte can be stimulated by bacterial products, complement fragments, or adhesion. Both of these cells circulate throughout the body, always vigilant for a specific antigen and an inflammatory nidus, respectively. This property allows these cells to search out their stimulus, as it were. As a sessile cell, however, the keratinocyte differs from the mobile cells noted above by an important criterion; the activating stimulus must come to the keratinocyte. Additional features distinguish the keratinocyte from mobile immune and inflammatory cells with regards to strategies of activation. There can be no question that a major role of the keratinocyte is to provide a physically tough, relatively impermeable barrier between host and environment; this is achieved by differentiation, cornification, and deposition of keratinocyte products in the upper epidermis. Thus, even though not "activated" with regards to cytokine production, the keratinocyte in the absence of cytokine inducing stimuli is growing and terminally differentiating. Unlike the "resting" T cell and monocyte, the "resting keratinocyte" is far from biologically inactive.

Why postulate the existence of an activated keratinocyte? The rationale lies in the significant potential for the keratinocyte to produce cytokines previously understood in the context of immune or inflammatory responses. Keratinocytes can be induced to synthesize and secrete IL-6, IL-8, GM-CSF, M-CSF, monocyte chemotactic factors, and less-well-characterized soluble activities with potent biologic effects [11–17]. If these secretory proteins were produced in appreciable quantities by normal epidermis, one would predict that leukocytes would be recruited and activated in situ, leading to a brisk inflammatory response. Experience and logic dictate that this

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is not the case. Indeed, to date we have been able to demonstrate convincingly only one biologically active cytokine in normal epidermis in the absence of disease; that is, interleukin-1-alpha [3,8,10]. Although it is presumed that in vivo this factor is produced by the epidermal keratinocyte, based upon immunohistochemical and in vitro evidence [3,4] it should be pointed out that this has not yet been unequivocally demonstrated. We have, however, made a working assumption that at least some of the IL-1-alpha in the epidermis is of keratinocyte origin. This stems in part from our observation that unlike other cells that secrete *biologically active* IL-1, the keratinocyte can produce biologically active IL-1-alpha but cannot normally convert inactive pro-IL-1-beta into a biologically active species [18]. Therefore, the presence of IL-1-alpha and the absence of IL-1-beta bioactivity in epidermis is presumptive evidence for a keratinocyte (rather than a bone marrow–derived cell) source.

A second important point is that neither IL-1-alpha nor -beta has a structure which permits its secretion from the cell by a conventional pathway [19]. The absence of a signal peptide, and the observation that in healthy keratinocyte cultures virtually all biologic activity is in a cell-associated fraction [5], implies that keratinocytes may simply be unable to actively secrete substantial amounts of IL-1. "Secretion" of IL-1-beta by the activated monocyte appears to be coupled to the monocyte-mediated enzymatic conversion of pro-IL-1 beta (the biologically inactive 33-kD species) to the mature, biologically active 17-kD species [20,21]. Because we have been unable to find conditions under which keratinocytes can productively process their pro-IL-1-beta (they appear to lack the monocyte IL-1 convertase enzyme; see [18]), it is not surprising that we cannot detect immunoreactive IL-1-beta outside of the cell. Similarly, IL-1-alpha from keratinocyte cultures is predominantly intracellular and composed of pro-IL-1-alpha [5,6,8]; however, both pro-IL-1-alpha and the mature molecule are biologically active (in contrast to IL-1-beta) [21]. Thus, it may indeed be the case that in vitro and in vivo keratinocytes cannot release significant amounts of IL-1 from a sequestered-cell–associated compartment.

Thermal, radiant, and kinetic energy can injure cells, and injured cells can become "leaky" with regards to intracellular proteins. These same injurious stimuli can also be lethal to cells, which then release their contents passively and completely. The consequence of killing a keratinocyte, then, would include the release of active pro-IL-1-alpha and inactive pro-IL-1-beta. The pro-IL-1-alpha could instantaneously bind to IL-1 receptors on target cells and mediate inducible events. By contrast, the pro-IL-1-beta released by the cell would require the action of inflammatory cell proteases to be converted to a mature biologically active species. Thus, the consequences of a superficial wound to skin would include the release of substantial amounts of active (pro-IL-1-alpha) and latent (pro-IL-1-beta) IL-1 from keratinocytes. The sequestration of a biologically active cytokine such as IL-1-alpha inside the cell is a powerful protective mechanism for the host that is particularly well suited to a tissue which comprises the first line of defense against the environment.

Can keratinocytes in skin respond to IL-1? Herein lies the potential raison d'etre of the activated keratinocyte. Of the keratinocyte cytokines listed above, the gene expression for each can be induced by exogenous IL-1 in vitro [11–17]. Thus, keratinocytes in culture, despite the fact that they contain IL-1, must express sufficient IL-1 receptors to respond to exogenously provided IL-1. Analysis of receptor density in cultured keratinocytes indicates that this is indeed the case; in fact, receptor numbers on keratinocytes can be regulated more significantly than on any cell type yet analyzed [7,8]. These observations indicate that the keratinocyte is poised to respond to IL-1 provided exogenously or by its injured neighbors.

One IL-1 receptor has been cloned and characterized [22–24]; it is present on T cells, fibroblasts, and epithelial cells (including keratinocytes). The relationship of the cell-surface expression of this receptor to IL-1 inducible responses was studied in an elegant series of experiments [25]. Chinese hamster ovary (CHO) cells, which express very few IL-1 receptors, were transfected with an expression vector containing the full-length IL-1 receptor cDNA. As predicted, the transfected cells showed large numbers of binding sites (roughly 5000 times more sites per cell than wild-type cells). When the responsiveness of these cells to exogenous IL-1 (CHO cells do not make biologically active IL-1) was determined by measuring the production of the IL-1 inducible cytokine G-CSF, the results were remarkable. Significantly less IL-1 was required to stimulate the transfected CHO cells to the same extent (as judged by G-CSF production) as the wild type or mock transfected CHO cells. In fact, it was calculated that roughly the same number of sites per cell needed to be occupied by ligand to induce an equivalent response. It should be pointed out that the IL-1R transfected cells responded (by G-CSF production) to quantities of IL-1 which were effectively "invisible" to control cells.

These results supported our previous hypothesis that it is the number of receptors occupied per cell, rather than the ratio of occupied to unoccupied receptors, which determine the level of response to IL-1 [3,11]. They also supported the concept of a "threshold level" of bound IL-1 required to activate a cell. By the mass action binding equation,

$$[\text{bound IL-1R}] = \text{Ka}\,[\text{free IL-1}][\text{unbound IL-1R}],$$

where Ka is the affinity constant for the IL-1R, [free IL-1] is the concentration of extracellular IL-1 accessible to the receptor, and [bound IL-1R] and [unbound IL-1R] are the numbers of occupied and unoccupied IL-1R, respectively. If total IL-1R = bound IL-1R + unbound IL-1R, then

$$\begin{aligned}[\text{bound IL-1R}] &= \text{Ka}\,[\text{free IL-1}]\,([\text{total IL-1R}] - [\text{bound IL-1R}]) \\ &= \text{Ka}\,[\text{free IL-1}]\,[\text{total IL-1R}] \\ &\quad - \text{Ka}\,[\text{free IL-1}]\,[\text{bound IL-1R}];\end{aligned}$$

therefore,

$$[\text{bound IL-1R}] + \text{Ka}\,[\text{free IL-1}]\,[\text{bound IL-1R}] = \text{Ka}\,[\text{free IL-1}]\,[\text{total IL-1R}],$$

$$[\text{bound IL-1R}]\,(1 + \text{Ka}\,[\text{free IL-1}]) = \text{Ka}\,[\text{free IL-1}]\,[\text{total IL-1R}],$$

and,

$$[\text{bound IL-1R}] = \frac{\text{Ka}\,[\text{free IL-1}]\,[\text{total IL-1R}]}{1 + \text{Ka}\,[\text{free IL-1}]}.$$

Cursory analysis of this equation predicts that if both Ka and [free IL-1] are held constant, the [bound IL-1R] varies directly with the [total IL-1R]. The Curtis et al experiments and our predictions are consistent with the existence of a [bound IL-1]*, defined as the minimal number of IL-1 receptors on a given cell or an average number on a population of cells that, if occupied with ligand, would result in a response (as measured by an IL-1 inducible event).

Further inspection of the above equation indicates that a given [bound IL-1R]* can be approached (assuming the constancy of Ka) by either increasing the [free IL-1] or the [total IL-1R].

Because the [free IL-1] variable is governed in vitro and (presumably) in epidermis by the capacity of IL-1 to leave its intracellular compartment (the issue of membrane-associated IL-1 and its possible contribution to the [free IL-1 compartment] is too speculative to address here), and because it seems likely that only injurious stimuli can influence this event, it may be that the more important variable in the equation is [total IL-1R] at least for pathophysiologic conditions not mediated by direct epidermal injury. Factors which can influence IL-1R expression have been discussed previously, and include phorbol esters, UVB irradiation, and T-cell cytokines. The spectrum of factors which work to increase or diminish IL-1R in vivo are essentially unexplored. However, in situ binding studies have indicated that normal epidermis expresses few detectable IL-1R [26].

It is postulated that, in normal uninjured epidermis, the product of [free IL-1] and [total IL-1R] is insufficient to allow the right side

of the equation to exceed hypothetical [bound IL-1R]*, that is,

$$[\text{bound IL-1R}]^* > \frac{\text{Ka [free IL-1] [total IL-1R]}}{1 + \text{Ka[free IL-1]}} \text{ (normal epidermis).}$$

Cells in this epidermis are, therefore (with regards to cytokine production only), "resting" keratinocytes. Because changes in either of the above variables should increase the value of the right side of the equation, it is of interest to examine what might influence each variable separately in purely hypothetical in vivo situations.

The most obvious variable with regards to [free IL-1] would be the release of IL-1 after injury to the skin. Traumatic injury would release preformed IL-1-alpha from its sequestered location. Within the epidermis, if this new [free IL-1] was sufficiently high that (with the ambient [total IL-1R]) a value on the right side of the equation which met or exceeded [bound IL-1R]* could be achieved, the result would be keratinocyte production of IL-1 inducible cytokines. Under the appropriate circumstances, this would lead to recruitment and activation of a leukocytic inflammatory infiltrate. It is important to add that the same principle of a "threshold of binding" required for activation applies to fibroblasts and endothelial cells; the difference, of course, is that the normal ambient [free IL-1] in the dermal microenvironment (in contrast to the epidermal microenvironment) approaches zero. Thus the traumatic introduction of epidermal interleukin-1 into the dermis will lead to the potential achievement of [bound IL-1R]* for fibroblasts and endothelial cells by increasing the [free IL-1] variable. It should be noted that whether the injury and subsequent release of IL-1 are instantaneous (trauma) or delayed (UVB irradiation), the net result should be similar.

Because dermal [free IL-1] is negligible, the independent up-regulation of [total IL-1] on fibroblasts and endothelial cells should be a null event in terms of IL-1 inducible cytokine production. However, because [free IL-1] in keratinocyte cultures (and epidermis?) is significant, the consequence of [total IL-1R] increases in keratinocyte cultures or epidermis may well lead to an analogous achievement of [bound IL-1]*, in a different manner. Because certain T-cell cytokines (to cite one example) can up-regulate IL-1R on keratinocytes, it is hypothetically possible that a T cell activated in or near the epidermis might induce keratinocyte cytokine production (and thus, indirectly, recruitment of an inflammatory infiltrate) by up-regulating [total IL-1R] in the face of ambient [free IL-1], thus achieving [bound IL-1R]*. It should be pointed out that although the concept of independent variation of [free IL-1] and [total IL-1R] is useful from a didactic perspective, most stimuli which enhance IL-1R expression also enhance IL-1 production. Important exceptions to this rule are retinoids, which enhance IL-1 production but block induction of IL-1R, and corticosteroids, which inhibit IL-1 production but are permissive for IL-1R induction [8,10]. The concept that the production of secondary cytokines can be regulated at the level of receptor expression for the inducing cytokine rather than the total quantity of the latter, has important implications for cutaneous biology.

The third potential variable is, of course, the value [bound IL-1R]*. It is completely unknown whether certain conditions or factors may influence this threshold value for IL-1-induced cellular activation, but this is entirely plausible. Regulation of the sensitivity of the cell to IL-1/IL-1R interactions alone may be sufficient to evoke an IL-1-inducible response, even in the absence of simultaneous changes in [free IL-1] and total IL-1R]. Because the post-receptor signaling events which follow interaction of IL-1 with its receptor are largely uncharacterized, regulation of [bound IL-1R]* is difficult to address rigorously. An even more difficult variable to address is, paradoxically, the hypothetical temporal variance of Ka (ostensibly a constant). However, there is ample evidence from other systems that the binding of ligand can facilitate subsequent ligand-receptor interactions on the same population of cells.

It should be pointed out that the model outlined above should apply to any cell capable of producing a cytokine to which it can respond (autocrine activation). The novel aspect of this model is the implication that receptor expression, rather than cytokine production, may be the principle determinant that governs the biologic response to a cytokine in vivo. For example, it has been postulated that a primary defect is psoriasis may be dysfunctional regulation of keratinocyte IL-1R in the absence of observable elevations in epidermal IL-1 [3,27]. This concept is also applicable to paracrine activation. Such observations and hypotheses make the analysis of the role of cytokines in human disease (and in particular skin disease) more complex. For example, the inability to observe a significant increase in mRNA or protein levels for a given cytokine in a given skin disease does not rule out that cytokine in the pathophysiology of that disease; rather, it is formally possible that an increase in expression of the specific receptor for that cytokine is a more important parameter.

Keratinocytes express receptors for many other cytokines previously understood in the context of immune and inflammatory events. Receptors for IFN-gamma are present on keratinocytes [27] and, unlike the IL-1R, normal epidermis constitutively expresses high levels of IFN-gamma receptors (R. Schreiber, personal communication). Thus, the epidermis appears to be vigilant for the possibility of T-cell activation near skin. The consequences of IFN-gamma binding to keratinocytes have been documented and include ICAM-1 expression and HLA-DR expression [29–31]. The IFN-gamma–stimulated keratinocyte may be a different variety of activated keratinocyte, because there is evidence that this cytokine also induces secondary cytokine production by keratinocytes. Other cytokine receptors are present on keratinocytes [3,14,32] and while the consequences of activation with these cytokines are less completely understood, it is possible that each "activates" the keratinocyte in a unique fashion (see Fig 1). If this is the case, a unique

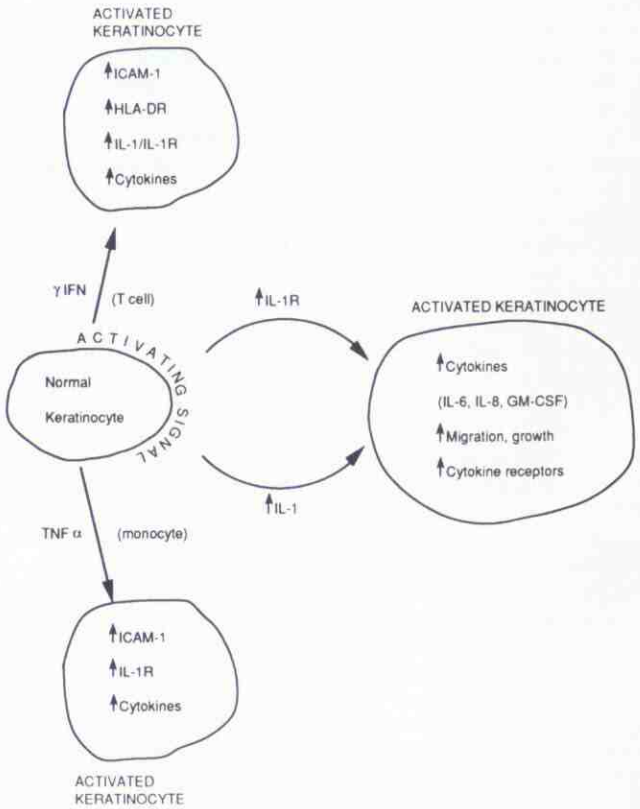

Figure 1. Generation of the activated keratinocyte from the "normal" or "resting" keratinocyte can occur as a result of autocrine or paracrine activation. Paracrine activation by T cells yields an activated keratinocyte which differs from one generated by products of monocytes. Activation of keratinocytes by IL-1 can occur as an autocrine or a paracrine event.

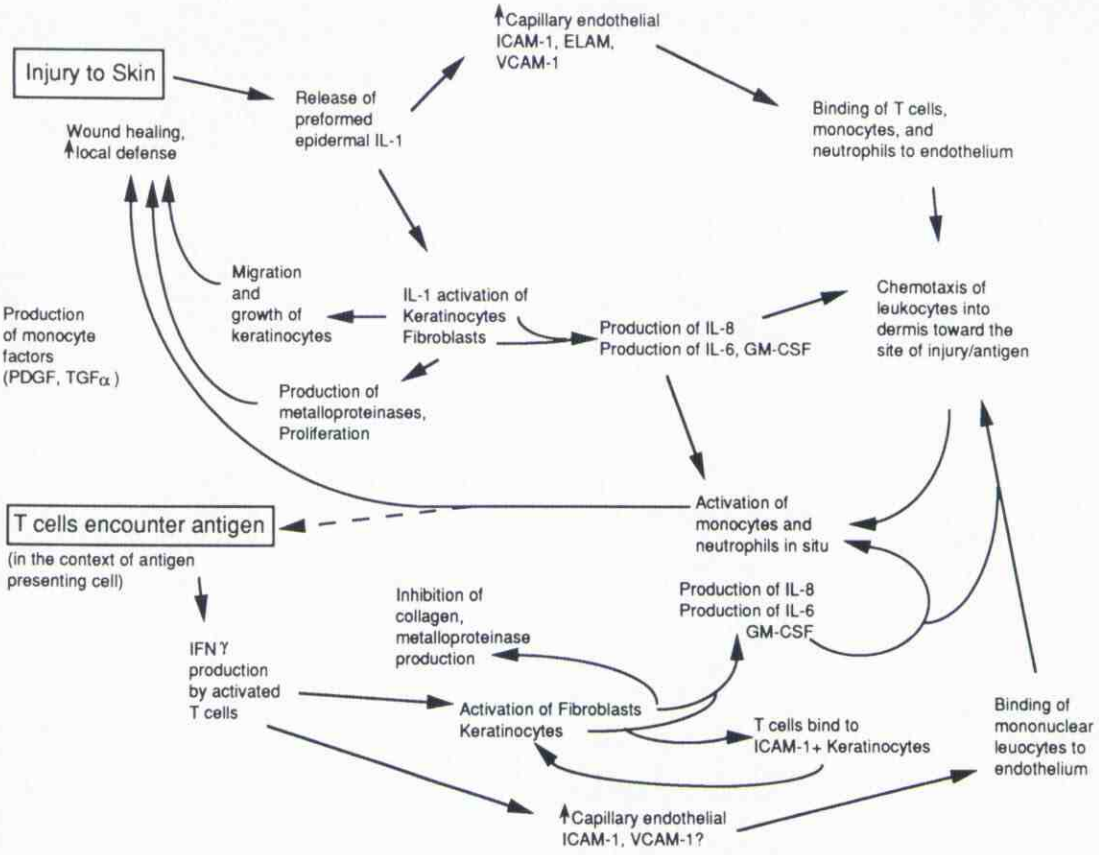

Figure 2. Hypothetical scheme which shows the role of the cytokine-activated keratinocyte or fibroblast in events following mechanical injury to skin or T-cell activation in skin. ICAM-1 [34], ELAM-1 [35], and VCAM-1 [36] are expressed on vascular endothelium in response to cytokine activation. Local production of cytokines by keratinocytes and fibroblasts serve to chemoattract (IL-8) and activate in situ (GM-CSF, IL-6, IL-8) leukocytes which have adhered to stimulated endothelium.

equation may be applicable to each, with the attendant complexities of variables of free ligand, bound ligand, [bound ligand]*, and Ka.

Biologic systems are complex and mutable, and tend to resist analysis by straightforward mathematical models. For example, we have not considered the participation of cytokine inhibitors in this model. Recent data suggests that one such inhibitor binds to the IL-1R with respectable affinity [33]; thus, yet another variable may need to be introduced into the equation above. The proposals and equations outlined above may ultimately be more instructive than predictive of inflammatory events in skin. Such analysis does, however, provide a foundation upon which further models can be constructed. It is also important to remember that with regards to cutaneous inflammation, cytokines are only part of the story, and that to effect migration of the leukocyte from its intracellular location through the vessel wall and to a specific site requires the expression of adhesion molecules by the leukocyte and resident tissue cells (Fig 2). The model of the activated keratinocyte, however, suggests that certain non-lymphoid tissues may both initiate and orchestrate inflammation, and that the epidermis is specifically poised to do so in response to signals from the external environment.

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