Perspectives

Epithelial tissues in general (and skin in particular) define boundaries between inside and outside, host and environment. Mammalian skin and its appendages are poised at the critical interface between the delicately balanced internal milieu of the host and the mercurial forces of the outside world. Human skin diseases characterized by histopathologically distinct patterns of infiltration by T cells, B cells, monocytes, and granulocytes (referred to collectively as leukocytes) number quite literally in the hundreds. To state that the pathophysiologic mechanisms involved in the evolution of such unique inflammatory infiltrates are poorly understood is to euphemize; yet, recent advances have begun to allow for the construction of hypothetical paradigms that address the issue of mechanism in cutaneous immune and inflammatory processes.

While skin was once considered the inert canvas upon which immune and inflammatory processes were painted by the bone marrow–derived host defense system, it is becoming apparent that resident sessile cells of skin are perhaps no less important in the generation of a cutaneous immune or inflammatory response. This contention is based upon two broad classes of observation. The first is that nonhematopoietically-derived cells resident to skin (including dermal fibroblasts, endothelial cells, and epidermal keratinocytes and melanocytes) can produce various cytokines upon appropriate stimulation (reviewed in reference 1) that affect decisively the behavior and function of lymphocytes, granulocytes, and monocytes. The second class of observation is that several of these cytokines influence the expression of adhesion molecules on endothelial and epithelial cells (1, 2). Taken to their logical conclusions, these observations suggest that nonhematopoietically-derived cells of skin, by virtue of their potential for cytokine production, have the indirect capacity to trap appropriate classes of leukocytes within microvasculature at precise anatomical sites, guide them through vessel walls and through dermal tissue along chemotactic gradients, and then activate them in situ. This article will review some of the evidence for this special immunocompetence of the skin, especially with regard to newer observations relating to cytokine production.

Cytokines in cutaneous tissues

It is axiomatic that cells of distinct lineages must communicate during the successful localisation and evolution of an inflammatory response, and cytokines comprise an important part of the language that facilitates this communication. Cytokines are small polypeptide molecules that interact with specific receptors and are produced by cells locally and transiently in response to exogenous stimulation. In general, cytokines mediate events relevant to immune and inflammatory processes. They include the interleukins (IL), the hematopoietic colony-stimulating factors (CSF), tumor necrosis factors alpha and beta, the interferons, and an assortment of the factors that, for various reasons, have not yet been grouped formally under an aegis (a process that is often less rational than it might ideally be) (1–4).

Table 1 summarizes the cytokines known to be produced in vitro by nonhematopoietically-derived cells (e.g., fibroblasts, keratinocytes, endothelial cells) normally resident to cutaneous tissue. It is important to recall that IL-6, IL-8, MCAF, and the hematopoietic CSFs are not produced by these cells in the absence of stimulation; they are inducible cytokines. Three cytokines appear to be especially important for the induction of their gene expression; notably, IL-1α, IL-1β, and TNFα. Based on their capacity to induce the production of so many secondary cytokines which have chemotactic and leukocyte-activating properties, as well as their direct effects on endothelial cell adhesion molecule expression (reviewed below), IL-1α and -β and TNFα may be termed “primary” or initiating cytokines for the purposes of this review. It now appears that production of certain primary cytokines antedates the phylogenetic appearance of antigen receptor–bearing cells (5), and that at least for an evolutionary window of time, putative cytokine-mediated activation of antigen receptor-negative leukocytes comprised a principal mode of cell-mediated host defense.

The biological activity first named interleukin 1 (IL-1) is composed of two molecules, IL-1α and β, whose genes (as well as at least one gene for the cellular receptor of both proteins) map to human chromosome two (1, 4, 6). The mature cytoplasmic mRNA transcripts of both IL-1 genes encode for 31-kD proteins, but whereas 31-kD IL-1 alpha binds to its receptor and is biologically active, 31-kD IL-1 beta is inactive until it is proteolytically processed to a 17-kD molecule. This processing is performed efficiently by monocytes, the cells from which the IL-1 cDNAs were originally cloned. The pleiotropic biological effects of the IL-1's have been the subject of many reviews and countless scientific reports, the scope and breadth of which has led recently to a sort of nihilism born of frustration with regard to assigning a true “biological role” to IL-1. Tempering this view is a body of evidence that suggests

1. Abbreviations used in this paper: CSF, colony-stimulating factor; ELAM-1, endothelial cell leukocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; Tcr, T cell receptor; VCAM-1, vascular cell adhesion molecule-1.

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Table I. Cytokines Reported to Be Produced In Vitro by Non-Bone Marrow-Derived Cells Resident to Skin (e.g., Fibroblasts, Endothelial Cells, Keratinocytes, Melanocytes

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 1 (IL-1) alpha</td>
<td>Monocyte, keratinocyte, epidermal cell, fibroblast, mast cell</td>
</tr>
<tr>
<td>IL-1 beta</td>
<td>Keratinocyte, epidermal cell, fibroblast, mast cell</td>
</tr>
<tr>
<td>IL-6*</td>
<td>Keratinocyte, epidermal cell, fibroblast, mast cell</td>
</tr>
<tr>
<td>IL-8/Neutrophil activating protein (NAP)-1*</td>
<td>Endothelial cell, M-CSF, IL-1,8/Neutrophil activating protein (NAP)-1*</td>
</tr>
<tr>
<td>Granulocyte/macrophage (GM)-colony-stimulating factor (CSF)*</td>
<td>Keratinocyte, monocyte, fibroblast, mast cell, endothelial cell</td>
</tr>
<tr>
<td>G-CSF*</td>
<td>Monocyte, keratinocyte, epidermal cell, fibroblast, mast cell</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Monocyte, keratinocyte, epidermal cell, fibroblast, mast cell</td>
</tr>
<tr>
<td>Monocyte chemotactic and activating factor (MCAF)*</td>
<td>Monocyte, keratinocyte, epidermal cell, fibroblast, mast cell</td>
</tr>
</tbody>
</table>

*Those cytokines whose gene expression in these cells can be induced by IL-1 and/or TNF alpha.

many of the diverse effects of IL-1 are indirect. For example, fibroblast proliferation induced by IL-1 appears to be mediated via production of PDGF induced by IL-1 (8), and gene expression for many of the hepatocyte-derived proteins which characterize the acute phase response are induced directly by IL-6 (9), another IL-1 inducible cytokine. Negative regulation of IL-1 might be expected to have widespread consequences, and the recent cloning of specific IL-1 inhibitor has generated broad-based interest (10).

Epidermis, the tissue closest to the outside world and the only tissue exposed to significant quantities of ultraviolet light, contains prodigious quantities of biologically active preformed IL-1 at rest (1, 11–13); in effect, we are covered by a shield of IL-1 that is continually being shed (by desquamation) and renewed. The source of this epidermal IL-1a is likely to be the keratinocyte, because these cells have been shown to produce IL-1a mRNA and protein in vitro (14). However, Langerhans cells and melanocytes have been shown to produce IL-1a in vitro. Biological activity attributable to IL-1β (the principle form of IL-1 produced by monocytes) cannot be demonstrated readily in either normal epidermis or in cultured keratinocytes (1, 13, 15, 15a). Interestingly, while the keratinocyte can make 31-kD IL-1 beta protein (which does not bind to the IL-1R and is devoid of significant biological activity), they cannot process it efficiently into a biologically active form (15a). So such processing is required for IL-1a, which is active in both 31-kD and 17-kD forms. The inability to process 31-kD IL-1β shown by keratinocytes and fibroblasts (despite their ability to make the protein upon stimulation) appears to derive from the absence of an IL-1 convertase enzyme which may be unique to monocytes (15, 16). 31-kD IL-1β produced by such cells must be cleaved by exogenous proteases to attain biological activity.

Few biologically relevant proteases can generate active IL-1 beta species, because IL-1β species generated by tryptic cleavage or elastase cleavage of the recombinant precursor have substantially less activity (15). In contrast, chymotryptic cleavage generates an IL-1β three amino acids longer than the authentic mature molecule and equally active in biological assays. We have demonstrated that latent or 31-kD keratinocyte IL-1β can be activated by chymotrypsin (15a), and that recombinant 31-kD IL-1β can be cleaved similarly by naturally occurring serine proteases with chymotryptic specificity, such as human cutaneous mast cell chymase (manuscript in preparation) and neutrophil cathepsin G. Many mesenchymal cells (e.g., fibroblasts, smooth muscle cells) appear to produce IL-1β as their predominant form upon activation, but appear to be unable to process the inactive 31-kD molecule (15a, 17).

It may be that processing and activation of latent IL-1β produced by these cells is accomplished in cutaneous tissues in a paracrine fashion by certain serine proteases provided by inflammatory cells. The consequence of these events will be to increase locally the amount of this important primary cytokine, whose ostensible role in cutaneous inflammation will be outlined presently.

Interleukin 1 and cutaneous inflammation

The presence of large amounts (in biological terms) of the active form of a potent pleiotropic cytokine like IL-1α in human epidermis is unlikely to be an evolutionary accident. We have recently demonstrated that keratinocytes, in addition to producing IL-1, express large numbers of specific IL-1 receptors (18, 19). This could be predicted by the observed response of these cells to IL-1 in vitro, but it is of interest that keratinocytes are able to regulate their expression of IL-1 receptors over a wider range than any other cell type thus far studied. Recent evidence that the degree of IL-1 receptor expression can regulate sensitivity of a cell to exogenous IL-1 (20), coupled with our recent demonstration that IL-1 receptors can be visualized in diseased epidermis (as well as organ cultures of normal epidermis), have led to the proposal of the "activated keratinocyte", a cell which by virtue of heightened expression of IL-1R and/or production of IL-1, can respond to IL-1 in autocrine or paracrine fashion (1). IL-1-stimulated keratinocytes have been shown (in vitro) to produce GM-CSF, IL-8, and IL-6, all of which might be expected to promote leukocyte infiltration in vivo (1, 18, 21–23). IL-1 also has growth promoting effects on keratinocytes that may be mediated via IL-1-inducible cytokines or changes in keratinocyte growth factor receptors (1, 24). The absence of such inflammation in normal skin suggests that in spite of large quantities of epidermal IL-1, interaction of this ligand with its receptor on the keratinocyte is somehow avoided. Indeed, it is difficult to demonstrate IL-1R on keratinocytes in normal skin.

Several lines of evidence suggest that if IL-1 is introduced into the dermis, a predictable series of events will occur. Injection of recombinant IL-1 into dermis in both mouse and man leads to the generation of a local inflammatory infiltrate (25, 26). Stratum corneum contains significant amounts of IL-1 (11, 27) that, if introduced into the dermis, similarly induces brisk inflammation. More recently, proteins extracted from both heel callus (thickened stratum corneum) as well as viable cells from forearm epidermis were purified by distinct strategies, and chromatographic fractions were injected into the skin of volunteers (26). Only those fractions that contained IL-1 activity by in vitro biological assays also induced inflammation in vivo. These observations would appear to confirm earlier speculations that if the epidermal shield of IL-1 is breached and its contents released into the dermis, IL-1-mediated events relevant to the localization and activation of a dermal infiltrate, as well as those relevant to epidermal and dermal wound healing, will ensue. Recently, the intriguing observation that human eccrine sweat contains IL-1 has been made (28); not only might this help explain high levels of IL-1 in stratum corneum, but it makes good sense from a teleological perspective. It would be highly adaptive to be able to rapidly
increase the quantity of IL-1 in one's epidermal shield under "fight or flight" conditions; these are not incidentally the same conditions that promote eccrine sweating. This latter example points to the probable role of the reservoir of IL-1 in epidermis; that is, to provide a ready-made package of this potent mediator in anticipation of traumatic disruption of skin (i.e., wounding).

Mechanisms of leukocyte recruitment

How might the process of IL-1-induced inflammation be addressed mechanistically? Circulating T cells, as well as other leukocytes, clearly can be recruited to skin during immune and inflammatory responses. Given our knowledge of the in vitro capabilities of various skin cells to produce cytokines, and given our understanding of the temporal sequence of events in cutaneous inflammation, we can begin to construct models for the recruitment and activation of an inflammatory cell infiltrate. The initial events must involve a modification of the luminal surface of the endothelial cell by the expression of molecules which mediate cell−cell adhesion, thereby allowing different classes of leukocytes to adhere. While grouping these proteins under the aegis "adhesion molecules" emphasizes their common properties, it is likely that cellular signals are transduced as a consequence of binding and that substantially different sequelae will follow in a given system depending on which adhesion molecule is engaged. Yet, for the purposes of this article, all such interactions can be viewed as immobilizing the leukocyte in time and space. Once immobilized, this leukocyte must now negotiate the endothelial cell layer and penetrate the basement membrane. After binding to and passing through the endothelial basement membrane, the leukocyte must migrate along a chemotactic gradient through a dense dermal matrix containing collagen, fibronectin, elastin, proteoglycans, and glycosaminoglycans. Whereas these poorly understood events will suffice for the induction of dermal inflammation, the migration of a T cell to the epidermis to interact with Langerhans cells or ICAM-1 positive keratinocytes requires the additional process of negotiating another basement membrane (at the dermal-epidermal junction) and migrating between keratinocytes connected by desmosomes and other cellular junctions. It is the contention of this review that these complex processes can be initiated and sustained to completion after the release of primary cytokines, including preformed epidermal IL-1 alpha.

Many vascular endothelial adhesion molecules relevant to leukocyte recruitment (with the exception of low levels of ICAM-1) are not expressed in undiseased tissue or on unstimulated cultured endothelial cells (1, 2, 29). mRNA accumulation, followed by cell surface protein expression, of such adhesion molecules can be induced by the primary cytokines, notably IL-1 and TNF alpha. This is true for ELAM-1, which binds to a cell surface determinant on neutrophils (2), VCAM-1, which binds to VLA-4 (a β 1 integrin) on T cells and other leukocytes (2, 29), and ICAM-1, which binds to LFA-1 (2, 29, 30), a widely distributed β 2 integrin. In the case of ICAM-1, expression can also be strongly induced by IFN-γ, produced by activated T cells. As noted above, traumatic injury to the skin sufficient to introduce preformed epidermal IL-1 into the papillary dermis would be expected to induce the expression of these molecules on endothelial cells in the superficial and deep dermal plexus. Thus, in the absence of any degree of preexistent monocyte or macrophage activation, cutaneous injury alone is sufficient in principle to initiate the requisite events (leukocyte-endothelial binding) for the evolution of an inflammatory or immune response.

How do leukocytes penetrate endothelial basement membranes to begin this directed migration through dermis? Based on in vitro evidence, it has been presumed that cells such as neutrophils and monocytes produce matrix degrading proteases upon binding to basement membranes (via laminin and collagen receptors?) essentially creating tunnels through which they can migrate. Elegant in vitro studies using basement membrane proteins synthesized by cultured endothelial cells provide evidence that neutrophils may indeed focally digest basement membranes before their migration through this matrix (31). T cells and monocytes, however, have not been studied extensively in analogous systems, and the matrix-degrading protease profile of activated T lymphocytes is a potentially fruitful area for further study. It may be that some cells can traverse basement membranes without utilizing proteolytic enzymes.

Once leukocytes have passed through the endothelial cell and basement membrane into perivascular tissue, they must find the proximal source of the signal which ultimately led to their focal adhesion to endothelial cells. Based largely on in vitro evidence, it is assumed that these cells migrate directionally along a gradient of increasing concentration of chemotactic factors. Such factors include IL-8 and MCAF, as well as the related family of peptides, several of which also cause activation of neutrophils and monocytes (4). As noted above, factors such as IL-1 and TNF α enhance the expression of IL-8 and MCAF by sessile cells in skin (e.g., fibroblasts and keratinocytes). The capacity of the same factor to both attract and activate a neutrophil or monocyte is an economical concept.

![Figure 1](Image)

**Figure 1.** Hypothetical scheme whereby injury to epidermis resulting in release of preformed IL-1α can lead to the localization of immune and inflammatory cells. It should be stressed that the "injury" need not be from kinetic trauma. Radiant injury, cytopathic virus infection, bacterial or fungal infection, or injury from chemical irritants may all lead to similar release of IL-1. In contrast to injury from trauma, these other injurious stimuli are unlikely to lead to blood vessel disruption, and thus the contribution of platelet-derived factors relevant to wound healing is avoided. The elegance of this hypothetical process is that memory T cells and other leukocytes are attracted in a precise fashion to the site of injury without the requirement for a systemic immune or inflammatory response.

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While a spherical shape might be well suited to a cell circulating in lymph or blood, migration through basement membranes and matrix-rich connective tissues is likely to require both cellular deformation and substantial mechanical forces to be exerted by the leukocyte. Thus, another important element of migration through tissue involves cytoskeletal mobilization in leukocytes. With this in mind, it should be noted that many cells, including leukocytes, express different members of a family of molecules known as integrins, heteromers composed of an \( \alpha \) and \( \beta \) chain (32). One integrin family combines a common \( \beta 1 \) chain (CD29) with one of six different \( \alpha \) chains (CD49a-f) in a heterodimeric complex. These heterodimers are known as \( \beta 1 \) integrins and are termed \( \alpha n\beta 1(n = 1-6) \) by convention; alternatively, they are called VLA molecules (29, 33), where VLA-\( n \) corresponds to \( \alpha n\beta 1 \). While most VLA molecules have been best described as cell-matrix protein adhesion molecules, others appear to mediate cell-cell binding as well. VLA-4 is the receptor for endothelial VCAM-1, as well as for a domain on the alternatively spliced V form of fibronectin (34). VLA-3, a receptor for collagen, laminin, and fibronectin, has also been proposed to moderate cell-cell interaction between keratinocytes through an unknown ligand (35).

LFA-1, the receptor for ICAM-1, is a member of the Leu-cam or \( \beta 2 \) integrin family that includes LFA-1 (CD11a/
CD18), Mac-1 (CD11b/CD18), and p150,95 (CD11c/CD18) (reviewed in reference 30). The cytoplasmic domain of both the β1 and β2 integrins chains is known to interact with the cytoskeleton via several intermediate molecules, and one would predict that binding of such integrins to their ligands under the appropriate conditions would result in not only adhesion but also altered (and presumably enhanced) motility. Such cytoskeletal activation after the binding of the leukocyte to the endothelial cell (for example) may prepare it for active transit through both basement membrane and perivascular dermal matrix. It should also be noted that fragments of collagen and fibronectin are chemotactic for certain leukocytes; cells responding to such fragments may have been induced to express high levels of activated integrin receptors for unique domains of fibronectin (VLA-4, VLA-5) and collagen (VLA-1, VLA-2). Finally, a nonintegrin molecule called CD44, formerly studied as a lymphocyte homing molecule mediating binding to high endothelial venules has been shown to be a hyaluronid receptor (36). Certain evidence suggests that CD44 binding also causes cytoskeletal changes.

**T cells and the skin**

Given their pivotal location, the fact that epithelial tissues are home to specialized subsets of T cells should come as no surprise. The observation that murine epidermis harbors a unique subset of T cells (as defined by type and homogeneity of Tcr rearrangement) identical to (and perhaps descended from) the earliest gamma/delta positive T cells in thymic ontogeny has attracted general interest in the immunological community (37). It has been postulated previously that such cells may have evolved to more efficiently deal with common cellular, bacterial, or viral antigens, a process which very likely antedated the evolution of the α/β T cell receptor. While these murine epidermal T cells do not have a direct homologue in man, a recent study analyzed the distribution of T cells in human skin (38). Of interest, there is a large population of alpha-beta Tcr-positive T cells which appear to be unique to epidermis and papillary dermis, most numerous in acral skin. Although CD4+ helper T cells have been thought to have a particular affinity for skin, most of the cells identified by this study were in fact CD4−/CD8+ and had a phenotype which was characteristic of previously activated or "memory" T cells (based on expression of surface markers). These observations, coupled with reports that indicate that a certain surface marker identifies T cells in infiltrates of skin but not of other organs (39), suggest that the old concept of "skin-associated lymphoid tissue" or SALT may have broader implications than originally thought. There may exist a phenotypically identifiable subset of T cells that normally recirculates to skin and mediates appropriate activities in that microenvironment.

Because there appear to be no mechanisms of recruiting classes of T cells into inflamed tissue based upon their antigenic specificity per se, it is likely that whole populations of circulating T cells (based upon adhesion molecule expression) are localized by the above process in tissue, with the expectation that at least some will possess an antigen receptor conferring appropriate antigenic specificity. Recent studies suggest that memory T cells (that is, T cells which have previously encountered antigen and have expanded in a clonal fashion) can be distinguished from naive T cells by their heightened expression of adhesion molecules such as LFA-1, CD29, LFA-3, CD44, and VLA-4, 5, and 6 (40). One would expect that such cells would more readily bind to endothelium and migrate more easily through connective tissue. In medicine, common things occur commonly, and by analogy, common antigenic pathogens are encountered commonly; thus, it makes good biological sense for memory T cells to be recruited preferentially by the non–antigen specific process outlined above.

Frank kinetic injury to skin is not the only means of releasing IL-1 from its epidermal location and recruiting memory T cells to skin. Burns from excessive ultraviolet B or ther-

![Figure 2 (Continued)](image-url)
Infiltration of molecular proper microbial cells of primary release cells will cause tissues cell sequelae. The nodes. Ultimately, skin. The molecules and subsequent consequences are clear that underlie various clinical consequences. It is expected that from the hypothesis are that active IL-1 is reduced in lesional psoriatic skin (which we interpret as consumption), and that IL-6 (an IL-1–inducible cytokine) is overexpressed in psoriatic skin (45). Whether such observations reflect cause and effect is as yet unknown; however, such perspectives offer new theoretical approaches to the analysis of skin diseases which have for years resisted successful analysis and therapy.

The accessibility of skin for scientific analysis means that in due course the models outlined above will be tested. New adhesion molecules and cytokines will undoubtedly be discovered; these may be integrated into the above paradigms or require wholesale revision of these perspectives. Yet, the process of leukocyte adhesion to endothelium, negotiation of basement membranes, and migration through perivascular connective tissue are processes that are universally applicable in inflammatory disease, whether the initiating stimulus is kinetin energy, immune dysfunction, or ischemia. Differences between analogous processes in different tissues are more likely to represent subtle variations on a highly conserved evolutionary theme than thoroughly tissue-specific phenomena with little in common. Thus, it is expected that from the study of skin may come important and previously elusive insights into general processes of human disease.

References


