The Interleukin-1 Axis and Cutaneous Inflammation

Thomas S. Kupper and Richard W. Groves

Division of Dermatology, Harvard Skin Disease Research Center, Harvard Medical School, Boston, Massachusetts, U.S.A.

Since the discovery that epidermal cell-derived thymocyte-activating factor was identical to interleukin (IL)-1α and -β in 1986, these molecules have been implicated in the pathogenesis of skin diseases. In 1995, it has become clear that a group of gene products function to regulate the activity of IL-1. IL-1α and mature 17-kD IL-1β (cleaved from precursor by IL-1β–converting enzyme) bind to the type 1 IL-1 receptor to transduce a signal. This process can be antagonized at the level of the receptor by two distinct forms of the IL-1 receptor antagonist, which bind to the type I receptor but do not transduce a signal. The process can also be antagonized at the level of the ligand by either cell-bound or soluble type 2 IL-1 receptor. This type 2 IL-1 receptor binds ligand but does not transduce a signal. Keratinocytes can make each of these variables *in vitro*, and the balance between agonists and antagonists dictates the biologic outcome of a putative IL-1–mediated event. Transgenic mice that overexpress each of these factors individually in epidermis will be useful for enhancing our understanding of the cutaneous biology of IL-1. *J Invest Dermatol 105:62S–66S, 1995*

Interleukin (IL)-1 is a pleiotropic proinflammatory cytokine that was defined as a "primary" cytokine based upon the prediction that its release, as an isolated event, would be sufficient to induce inflammation. In the past several years, it has become clear that there are multiple ligands for IL-1 receptors, some of which are agonists and others of which are antagonists. In addition, a non–signal-transducing receptor that efficiently binds IL-1 agonists has been identified **(Table I)**. These multiple molecules form the IL-1 family of agonists and antagonists. Much of this report will detail the *in vitro* and *in vivo* regulation of IL-1 family members that are believed to influence the outcome of a putative IL-1–mediated event. For the purposes of this discussion, an IL-1–mediated event is defined as a consequence of the interaction of an active IL-1 species (defined below) with its signal-transducing (type I) receptor. Before these issues are analyzed in detail, it is instructive to discuss precisely how IL-1 so efficiently localizes inflammation. The putative sequence of events following the release of keratinocyte IL-1 into the epidermis and dermis has been discussed in detail previously and is outlined below.

IL-1 is a potent inducer of endothelial adhesion-molecule expression [1–4], leading to endothelial cell surface expression of P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1). The *de novo* expression of endothelial selectins, and their interaction with counter-receptors on leukocytes, are now accepted as important initial requirements for leukocyte extravasation [5]. Several groups have now demonstrated that neutrophils and myelomonocytic cell lines can bind and roll on monolayers of selectin-expressing endothelial cells at flow rates that approximate flow through post-capillary venules [6]. Very recently, we have demonstrated that T cells can roll on endothelial selectins [7]. Whether T cells (or other cells expressing very late activation antigen 4 [VLA-4]) can bypass the requirement for selectins is unclear, as recent reports have suggested that certain integrin/CAM interactions may be sufficient for initial selectin-independent tethering interactions [8]. This process would, however, involve VLA-4/VCAM-1 interactions and thus is still influenced by IL-1.

The attachment and rolling under conditions of flow are required for subsequent events leading to extravasation [5]. The first of these events involves activation of the leukocyte, which is achieved *via* the interaction of chemokines produced locally in tissue with their receptors on leukocytes. These chemokines (e.g., monocyte chemotactic factor 1 [MCP-1], IL-8, gro-α; reviewed in [9]), all of which are induced by IL-1 *in vitro*, can both activate the leukocyte at the vessel wall [5] and provide a chemotactic gradient such that when the leukocyte extravasates, it will be guided toward the original site of injury (i.e., the original site of IL-1 release). It now appears that chemokines bind avidly to glycosaminoglycans [5,10], such that immobilized chemokines can decorate the luminal aspect of the endothelium in a perfect position to activate leukocytes rolling on endothelial selectins. The gradient formed in the dermis can be fixed in place *in situ* by virtue of chemokine binding to dermal glycosaminoglycans, and this gradient can persist in the absence of continued chemokine production by injured cells.

After the leukocyte has slowed to a roll on endothelial selectins and has been activated by locally produced chemokines, it will bind *via* VLA-4 or leukocyte-function associated antigen-1 (depending on cell type and circumstances) to endothelial VCAM-1 and ICAM-1 or -2, respectively [5]. The cytoplasmic domains of these integrins are linked to the actin cytoskeleton of the leukocyte [11], and in response to chemokine-mediated gradients, the leukocyte will insinuate itself between endothelial cells, cross the endothelial basement membrane, and emerge into the dermis on the abluminal side of the vessel. Given that IL-1 can orchestrate these events, it would appear potentially dangerous for nature to place constitutively high levels of IL-1α in the epidermis at all times. Recent advances in understanding the regulation of IL-1 activity underscore that this is one of the most highly regulated cytokine systems known **(Fig 1)**.

Reprint requests to: Harvard Skin Disease Research Center, Harvard Medical School, Division of Dermatology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115.

0022-202X/95/$09.50 • SSDI0022-202X(95)00210-C •

Table I. Agonists and Antagonists of IL-1–Mediated Biologic Events

Agonists	Antagonists
IL-1α (17 kD, 31 kD)	IL-1 receptor antagonist
IL-1β (17 kD)	Intracellular (icIL-1ra)
	Secreted (sIL-1ra)
IL-1 receptor (type 1)	IL-1 receptor (type 2)
	Cell surface
	Soluble

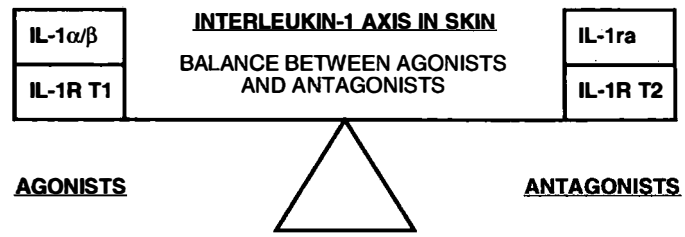

Figure 1. Interleukin 1 axis in skin.

BIOLOGIC FORMS OF IL-1

IL-1α and -β are both transcribed from genes on chromosome 2 in man and in mouse [12,13] and are translated as 31-kD proteins lacking a leader sequence and signal peptide [14,15]. It appears that they are not glycosylated (clearly they do not enter the Golgi complex or the rough endoplasmic reticulum; see [15,16]), and post-translational modification appears to consist largely of myristoylation for IL-1α [17] and proteolytic cleavage with subsequent activation for IL-1β [18,19]. Although both 31-kD and processed 17-kD IL-1α bind to the IL-1 receptor 1 (IL-1R1) and transduce signal [20], only 17-kD (and not 31-kD) IL-1β binds and transduces a signal [20]. The proteolytic cleavage of IL-1β is cell-type specific [19,21]; monocytes contain an enzyme (IL-1β–converting enzyme) that cleaves 31-kD IL-1β to form an N-terminal Ala-117 IL-1β [21]. Although keratinocytes do not contain this enzyme activity and thus cannot process 31-kD IL-1 intracellularly [22], release of 31-kD IL-1β into a milieu containing proteases with chymotryptic specificities (e.g., mast cell chymase or cathepsin G) leads to generation of an active N-terminal Val-114 species of IL-1β [22].

The precise mechanism of release of IL-1 from cells remains enigmatic and has been incompletely explained [16,23]. Like basic fibroblast growth factor, IL-1 is released most completely from cells that are physically disrupted. However, there is growing evidence for the existence of alternative secretion mechanisms for molecules like IL-1α, IL-1β, and basic fibroblast growth factor that lack a signal peptide [24–26]. These are phylogenetically ancient transport pathways, and homologues of the pathways can be found in bacteria. Thus, the hypothesis that IL-1 will only exit cells as a result of cell death may no longer be tenable [12–14]. Under certain circumstances, IL-1α may be localized to the cell membrane by protein myristoylation [17,27], although whether the membrane-associated product is oriented in such a way that it can interact with the IL-1R1 on adjacent cells is speculative. Cells that contain the IL-1β–converting enzyme (e.g., monocytes) appear to be able to release the 17-kD form of IL-1β readily, although the precise transport mechanism has not been identified [16]. A recent report suggested that cells undergoing apoptosis acquire the capacity to release IL-1 more efficiently before cell death [28]. The precise transport pathway that permits IL-1 "secretion" from cells is the subject of intensive study, but there is no longer serious doubt that such a pathway exists [23].

THE IL-1 RECEPTOR ANTAGONIST (IL-1ra)

A unique molecule first cloned and characterized in 1990 functions as a pure antagonist of biologically active IL-1 [29,30]. The IL-1ra gene resides near genes encoding for IL-1α and -β on chromosome 2 in man [31] and bears certain homology to these molecules. However, the IL-1ra binds to the IL-1R1 nearly irreversibly without triggering signal transduction [32,33], thus temporarily removing the bound receptor from subsequent participation in IL-1–mediated signal transduction. The form of the IL-1ra first described was a product of monocytes [34], and it is the only ligand for the IL-1R that can be efficiently secreted from cells. This secreted IL-1ra, or sIL-1ra, contains a leader sequence and signal peptide, is post-translationally modified in the Golgi complex, and is released by cells through a classic secretion pathway [34,35]. It does not require post-translational proteolytic modification to acquire binding activity [31]. This is the only "cytokine" known to function as a pure antagonist without any identifiable partial agonist function [31].

After the discovery of the sIL-1ra, it was noted that keratinocytes and other epithelial cells contained an 18-kD intracellular IL-1 inhibitory activity that was not efficiently secreted [36,37]. This activity was later identified as an alternatively spliced product of the same IL-1ra gene that generates sIL-1ra [37]. Called intracellular IL-1ra, the 5′ end of mature mRNA from this species arises from the 3′ end of exon 1, several kilobase pairs upstream from the exon 2 start site of sIL-1ra [38]. This alternative splicing of the parent transcript ultimately generates a protein that contains an additional seven N-terminal amino acids and lacks the signal peptide and leader sequence intrinsic to sIL-1ra [37]. Thus, this 18-kD molecule resembles IL-1α and -β in that it resides in a cell-associated compartment, lacks a leader sequence and signal peptide, and cannot be secreted by conventional pathways. In addition, it is not glycosylated or otherwise post-translationally modified [38]. It is likely to be released by the same nonclassic transport pathway as IL-1α and -β [16,23].

The presence of both IL-1α and the intracellular IL-1ra inside keratinocytes seems to represent a paradox; after all, when a keratinocyte is either induced to secrete or is lysed, it releases both agonist and antagonist, and the biologic consequences are unpredictable. Indeed, the wisdom of this arrangement remains elusive. However, the very fact that epidermal cell-derived thymocyte-activating factor/IL-1 activity was discovered at all in keratinocyte cultures, where antagonist is more abundant than agonist, and the multiple demonstrations that epidermis contains measurable bioactive IL-1α, indicate that regardless of the outcomes predicted by molar ratios, the agonist effect of IL-1 clearly dominates. In some systems, the ratio of antagonist to agonist (or IL-1/IL-1ra) must be greater than 100:1 for complete blockade of IL-1–inducible events [39], but partial inhibition is seen at lower ratios [39,40]. It is likely that although biologically available quantities of the IL-1ra may be insufficient to block completely IL-1–inducible events *in vivo*, the presence of IL-1 and IL-1ra may modify the strength of IL-1–inducible responses. There is no doubt, however, that pharmacologic doses of the IL-1ra can strongly inhibit IL-1 activity [41,42], and it is difficult to imagine that the physiologic role of IL-1ra does not involve homeostasis of the IL-1 axis [39,43].

THE IL-1 RECEPTORS

The IL-1R1 is the sole known signal-transducing receptor for IL-1 [44,45]. It is a variably glycosylated 80-kD molecule with a cytoplasmic domain of 215 amino acids [44]. It appears that all cells that respond to IL-1 express the IL-1R1, based on blocking experiments performed with neutralizing IL-1R1 and IL-1R2 antibodies [44–46]. The mechanism of IL-1–mediated signaling is currently the subject of intense study.

In general, ligand activation of receptors is assumed to occur either by induced receptor aggregation or by ligand-binding–related conformational changes transmitted by the extracellular and transmembrane parts of the receptor to the cytoplasmic domain. A considerable body of evidence suggests that IL-1–induced signal transduction occurs by the second mechanism. Cross-linking experiments have not demonstrated receptor aggregation such as that

seen with epidermal growth factor or platelet-derived growth factor receptors in similar studies [47,48]. Furthermore, anti–IL-1R1 antibodies showed no capacity to induce responses in cells sensitive to IL-1. Finally, as few as one to 10 IL-1 molecules bound per cell are required to trigger signaling [49–51], an unusually efficient mechanism that would be unlikely to result from aggregation of widely spaced receptors. The structure of the receptor itself [46,52,53] gives little clue as to the mechanism of action. For example, the cytoplasmic domain bears no resemblance to known protein kinases [54], and immunoprecipitated receptor shows no protein kinase activity [55]. Transfection experiments have demonstrated that the cytoplasmic domain is required for receptor function [56], and the IL-1R2, which has a truncated cytoplasmic domain, does not transduce a signal. A variety of signaling pathways have been implicated in the IL-1 response, including arachidonic acid [57–61], protein kinase C [62,63], cyclic adenosine monophosphate [64,65], and ceramide [66], but in many cases the evidence is conflicting.

Very recent data have suggested that the IL-1R1 activates a novel protein kinase cascade that results in the phosphorylation of a variety of proteins, including the 27-kD heat shock protein HSP27 [67–69]. The mechanism underlying this has now been studied in some detail, and it is apparent that three enzymes of MW 35, 40, and 50 kD are sequentially phosphorylated; the latter two have been purified to near homogeneity. The system closely resembles, but is molecularly distinct from, the microtubule assembly protein kinase cascade [70], and the amplification that likely results from sequential activation of these kinases is likely to contribute to the exquisite sensitivity of the IL-1 response.

The IL-1R2 encodes a 68-kD molecule that was initially cloned and characterized on B cells and hematopoietic cells [71–74]. It binds to IL-1β but does not appear to transduce a signal [44,45]. It is now clear that this receptor is much more widely distributed and is present on epithelial cells, including keratinocytes [75,76]. The most revealing feature of this receptor is its short cytoplasmic tail (29 amino acids) and the lack of any evidence that it transduces a signal [44,45]. Unlike the IL-1R1, which cannot be regulated efficiently [75–77], the IL-1R2 can be regulated dramatically by various stimuli [78,79]. Its function appears to be to antagonize the biologic effects IL-1. Of note, it can be shed very efficiently from cells including keratinocytes [76,80]. Intuitively, it would seem that this shed receptor would be significantly inhibitory to IL-1–mediated responses. Additional evidence supporting this notion derives from observations that vaccinia and cowpox viruses produce factors homologous to the IL-1R2 [81,82]. These soluble receptor-like molecules effectively bind to human IL-1β and inhibit IL-1–mediated inflammation [82]. It was proposed that viruses have exploited this product to reduce host defenses after their initial infection, thus favoring establishment [82].

Although much work has been done on the regulation of ^{125}I–IL-1 ligand binding to cells, including keratinocytes, many of these studies were performed before it became clear that there were two distinct IL-1 receptors, and the vast majority were not interpreted against the background of recent evidence that IL-1R2s are nonfunctional [44,45,83–85]. It is clear from multiple experiments, however, that enhanced expression of IL-1R1 can lead to increased sensitivity to extracellular IL-1 [86,87]. One study in particular indicated that transfection of IL-1R1 into cells deficient in this receptor rendered them responsive to quantities of IL-1 three orders of magnitude lower than quantities of IL-1 to which the wild-type cells responded [86]. Many cells in which large increases of IL-1R were demonstrated after stimuli showed up-regulation of IL-1R2s, or at least a combination of IL-1R1 and IL-1R2 [75,76]. One exception is the illustration that platelet-derived growth factor can up-regulate IL-1R levels fivefold on fibroblasts [87]; these cells probably do not express IL-1R2s [88,89] and thus this variation can be reliably attributed to IL-1R1. Very recently, cloning and analysis of the promoter region of the IL-1R1 were accomplished [77]. Surprisingly, the promoter resembled that of a number of so-called "housekeeping" genes, suggesting that regulation of this receptor is likely to be limited. Indeed, we have found that stimuli that up-regulate specific ^{125}I–IL-1 binding to human keratinocytes change IL-1R2 expression dramatically and IL-1R1 expression less so [76]. It appears that much more energy is spent preventing responses to IL-1 at the level of the receptor than allowing them to occur more efficiently. Thus, antagonists of IL-1 and non–signal-transducing receptors (IL-1R2) are both produced most abundantly by cells that also produce IL-1 [91].

A primary function of IL-1R2s is to be shed from cells to serve as a soluble scavenger of active IL-1 as well as pro–IL-1β molecules [76,80,82]. It is not difficult to understand the relevance of this factor to the epidermal microenvironment [2,90]. It would seem intuitively correct that, if cell surface IL-1R1 remained constant, concurrent high-level expression of IL-1R2 on the same cells would make them less sensitive to activation by a constant level of extracellular IL-1. Preliminary studies have shown that antibodies that block IL-1R2 binding do enhance the responsiveness of keratinocytes to IL-1. A working hypothesis, then, is that whether shed or retained on the cell surface, IL-1R2s have a generally inhibitory activity.

Since the discovery of keratinocyte-derived IL-1 [91,92], hypotheses about the role of IL-1 in skin disease have abounded. Increased or decreased levels of different members of the IL-1 axis have been reported in cutaneous T-cell lymphoma [93], contact hypersensitivity [94,95], acne vulgaris [96], scleroderma [97], human immunodeficiency virus-1–associated skin diseases [98], graft versus host disease [99,100], Kawasaki disease [101], melanoma [102], epidermal carcinogenesis [103,104], and psoriasis vulgaris [75,105–107]. It is important to stress that these studies report correlations between levels of individual IL-1 family molecules and clinical/histopathologic diagnoses. The relation of IL-1 and related molecules (or other cytokines, for that matter) to the pathogenesis of any of these diseases remains hypothetical.

TESTING THE PRIMARY CYTOKINE HYPOTHESIS USING TRANSGENIC MICE

The advantages of using transgenic mice to prove hypotheses about the authentic role of proinflammatory molecules in skin are substantial. Using promoters that direct the expression of transgenes in a tissue-specific fashion, it is possible to overexpress individual molecules in particular tissues. A series of transgenic mice from our laboratories have been made using the keratin-14 promoter. These studies have shown that expression of adhesion molecules such as ICAM-1 [108] or costimulatory molecules such as B7-1 [109] are not sufficient to induce inflammation. Similarly, overexpression of the chemokines MCP-1 and gro-α in basal epidermis does not provoke spontaneous inflammation [110], though such chemokines can alter the course of elicited inflammation in a predictable fashion. However, transgenic mice that overexpress the primary cytokines IL-1α and tumor necrosis factor-α in basal epidermis do develop inflammation and skin disease, thus providing *in vivo* proof that primary cytokines are sufficient to induce inflammation. A series of additional transgenic mice have been constructed that overexpress each member of the IL-1 axis in basal epidermis. We predict that mice that overexpress the IL-1ra and the IL-1R2 in basal epidermis will resist IL-1–mediated inflammation, whereas mice that overexpress the IL-1R1 will be more sensitive to IL-1. Although preliminary results suggest that these predictions are correct, these transgenic lines must be analyzed much more extensively before definitive statements can be made about IL-1 family members in the epidermis.

REFERENCES

1. Kupper TS: Immune and inflammatory processes in cutaneous tissues. Mechanisms and speculations. *J Clin Invest* 86:1783–1789, 1990
2. Groves RW, Ross E, Barker JNWN, Ross J, Camp CDR, MacDonald DM: Effect of in vivo interleukin 1 on adhesion molecule expression in normal human skin. *J Invest Dermatol* 98:384–387, 1992
3. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL

1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J Immunol* 137:245–254, 1986
4. Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B: Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. *Science* 250:1132–1135, 1990
5. Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76:301–314, 1994
6. Lawrence MB, Springer TA: Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell* 65:859–873, 1991
7. Alon R, Rossiter H, Wang X, Springer TA, Kupper TS: Distinct cell surface ligands mediate T lymphocyte attachment and rolling on P and E selectin under physiological flow. *J Cell Biol* 127:1485–1495, 1994
8. Berlin C, Bargatze RF, Campbell J: Alpha 4 integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell* 80:413–421, 1995
9. Oppenheim JJ, Zachariae COC, Mukaida N, Matsushima K: Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu Rev Immunol* 9:617–648, 1991
10. Tanaka Y, Adams D, Hubscher S, Hirano H, Siebenlist U, Shaw S: T cell adhesion induced by proteoglycan immobilized cytokine MIP-1 beta. *Nature* 361:79–81, 1993
11. Hynes RO: Integrins: versatility, modulation, and signalling in cell adhesion. *Cell* 69:11–25, 1993
12. Dinarello CA: Interleukin 1 and interleukin 1 antagonism. *Blood* 77:1627–1652, 1991
13. Dinarello CA, Wolff SM: The role of interleukin-1 in disease. *N Engl J Med* 328:106–113, 1993
14. March C, Mosely B, Larsen A, Cerretti D, Braedt G, Price V, Gillis S, Henney C, Kronheims S: Cloning sequence and expression of two distinct human interleukin-1 complementary DNA's. *Nature* 315:641–647, 1985
15. Auron PE, Webb AC, Rosenwasser LJ, Mucci SF, Rich A, Wolff SM, Dinarello CA: Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc Natl Acad Sci USA* 81:7907–7911, 1984
16. Stevenson FT, Torrano F, Locksley RM, Lovett DH: IL-1: the patterns of translation and intracellular distribution support alternative secretory mechanisms. *J Cell Physiol* 152:223–231, 1992
17. Stevenson FT, Bursten SL, Fanton C, Locksley D, Lovett DH: The 31-kDa precursor of IL-1 alpha is myristoylated on specific lysines within the 16-kDa N-terminal propiece. *Proc Natl Acad Sci USA* 90:7245–7249, 1993
18. Black R, Kronheim S, Sleath P, Greenstreet T, Virca GD, March C, Kupper TS: The proteolytic activation of interleukin-1 beta. *Agents Actions* 35:85–89, 1991
19. Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet T: Molecular cloning of the interleukin-1 beta converting enzyme. *Science* 256:97–100, 1992
20. Mosley B, Urdal D, Prickett K, Larsen SA, Cosman D, Conlon P, Gillis S, Dower S: The interleukin-1 receptor binds the human interleukin-1a precursor but not the interleukin-1b precursor. *J Biol Chem* 262:2941–2944, 1987
21. Thornberry NA, Bull HG, Calaycay JR, Chapman K, Howard A, Kostura M: A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* 356:768–774, 1992
22. Mizutani H, Black R, Kupper TS: Human keratinocytes produce but not process pro-IL-1 beta: different strategies of IL-1 production and processing in monocytes and keratinocytes. *J Clin Invest* 87:1066–1071, 1991
23. Rubartelli A, Bajetto A, Allavena G, Cozzolino F, Sitia R: Post-translational regulation of IL-1 beta secretion. *Cytokine* 5:117–124, 1993
24. Wandersman C, Delepelaire P, Letoffe S, Ghigo J: A signal peptide-independent protein secretion pathway. *Antonie Van Leeuwenhoek* 61:111–113, 1992
25. Kuchler K, Thorner J: Secretion of peptides and proteins lacking hydrophobic signal sequences. *Endocr Rev* 13:499–514, 1992
26. Mignatti P, Rifkin DB: Release of basic fibroblast growth factor, an angiogenic factor devoid of secretory signal sequence. *J Cell Biochem* 47:201–207, 1991
27. Bursten SL, Locksley RM, Ryan JL, Lovett DH: Acylation of monocyte and glomerular mesangial cell proteins. *J Clin Invest* 82:1479–1488, 1988
28. Hogquist KA, Nett MA, Unanue ER, Chaplin DD: Interleukin 1 is processed and released during apoptosis. *Proc Natl Acad Sci USA* 88:8485, 1991
29. Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Hemdal PL, Armes LG, Sommer A, Eisenberg SP, Thompson RC: Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 343:336–340, 1990
30. Eisenberg SP, Evans RJ, Arend WP, Verdeiber E, Brewer MT, Hannum CH, Thompson RC: Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature* 343:341–346, 1990
31. Arend WP: Interleukin-1 receptor antagonist. *Adv Immunol* 54:167–227, 1993
32. Dripps DJ, Brandhuber BJ, Thompson RC, Eisenberg SP: Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 266:10331–10336, 1991
33. Arend WP, Coll BP: Interaction of recombinant monocyte-derived interleukin-1 receptor antagonist with rheumatoid synovial cells. *Cytokine* 3:407–413, 1991
34. Arend WP, Joslin FG, Massoni RJ: Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor. *J Immunol* 134:3868–3875, 1985
35. Arend WP, Joslin FG, Thompson RC, Hannum CH: An IL-1 inhibitor from human monocytes. Production and characterization of biologic properties. *J Immunol* 143:1851–1858, 1989
36. Bigler CF, Norris DA, Weston WL, Arend WP: Interleukin-1 receptor antagonist production by human keratinocytes. *J Invest Dermatol* 98:38–44, 1992
37. Haskill S, Martin G, Van Le L: cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. *Proc Natl Acad Sci USA* 898:3681, 1990
38. Steinkasserer A, Spurr NK, Sim RB: Chromosomal mapping of the human IL-1 receptor antagonist gene (IL-1RN) and isolation of specific YAC clones. *Agents Actions* 38(Special Conf. Issue):C59–C60, 1993
39. Arend WP, Welgus HG, Thompson RC, Eisenberg SP: Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest* 85:1694–1697, 1990
40. Dripps DJ, Brandhuber BJ, Thompson RC, Eisenberg SP: Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 266:10331–10336, 1991
41. Mancilla J, Garcia P, Dinarello CA: The interleukin-1 receptor antagonist can either reduce or enhance the lethality of Klebsiella pneumoniae sepsis in newborn rats. *Infect Immun* 61:926–932, 1993
42. Porat R, Poutsiaka DD, Miller LC, Granowitz EV, Dinarello CA: Interleukin-1 (IL-1) receptor blockade reduces endotoxin and Borrelia burgdorferi-stimulated IL-8 synthesis in human mononuclear cells. *FASEB J* 6:2482–2486, 1992
43. Krzesicki RF, Hatfield CA, Bienkowski MJ, McGuire JC, Winterrowed GE: Regulation of expression of IL-1 receptor antagonist protein in human synovial and dermal fibroblasts. *J Immunol* 150:4008–4018, 1993
44. Sims J, Gayle M, Slack J, Alderson M, Bird T, Giri J, Colotto FR, Mantovani A: Interleukin 1 signaling occurs exclusively via the type 1 receptor. *Proc Natl Acad Sci USA* 90:6155–6159, 1993
45. Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Strone M, Giri JG, Dower SK, Sims JE, Mantovani A: Interleukin 1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* 261:472–475, 1993
46. Kuno K, Okamoto S, Hirose K, Murakami S, Matsushima K: Structure and function of the intracellular portion of the mouse IL-1R type I. *J Biol Chem* 268:13510–13518, 1993
47. Cochet C, Kashles O, Chambaz EM, Borrello I, King CR, Schlessinger J: Demonstration of epidermal growth factor-induced receptor dimerization in living cells using a chemical covalent cross-linking agent. *J Biol Chem* 263:3290–3295, 1988
48. Heldin CH, Ernlund A, Rorsman C, Ronnstrand L: Dimerization of B-type platelet-derived growth factor receptors occurs after ligand binding and is closely associated with receptor kinase activation. *J Biol Chem* 264:8905–8912, 1989
49. Dower SK, Call SM, Gillis S, Urdal DL: Similarity between the interleukin 1 receptors on a murine T-lymphoma cell line and on a murine fibroblast cell line. *Proc Natl Acad Sci USA* 83:1060–1064, 1986
50. Hall DJ, Brownlee C, Stiles CD: Interleukin-1 is a potent regulator of JE and KC gene expression in quiescent BALB/c fibroblasts. *J Cell Physiol* 141:154–159, 1989
51. Orencole SF, Dinarello CA: Characterization of a subclone (D10S) of the D10-G4.1 helper T-cell line proliferates to attomolar concentrations of IL-1 in the absence of mitogens. *Cytokine* 1:14–22, 1989
52. Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM, March CJ, Dower SK: Cloning the IL-1 receptor from human T cells. *Proc Natl Acad Sci USA* 86:8946–8950, 1989
53. Sims JE, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan CJ, Grubin CE, Wignall JM: cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science* 241:585–589, 1988
54. Hanks SK, Quinn AM, Hunter T: The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* 241:42–52, 1988
55. Gallis B, Prickett KS, Jackson J, Slack J, Schooley K, Sims JE, Dower SK: IL-1 induces rapid phosphorylation of the IL-1 receptor. *J Immunol* 143:3235–3240, 1989
56. Curtis BM, Gallis B, Overell RW, McMahan CJ, DeRoos P, Ireland R, Eisenman J, Dower SK, Sims JE: T cell interleukin 1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to IL-1. *Proc Natl Acad Sci USA* 86:3045–3049, 1989
57. Chang J, Gilman SC, Lewis AJ: Interleukin 1 activates phospholipase A2 in rabbit chondrocytes: a possible signal for IL-1 action. *J Immunol* 136:1283–1287, 1986
58. Gilman SC, Chang J, Zeigler PR, Uhl J, Mochan E: Interleukin-1 activates phospholipase A2 in human synovial cells. *Arthritis Rheum* 31:126–130, 1988
59. Suffys P, Van Roy F, Fiers W: Tumor necrosis factor and interleukin 1 activate phospholipase in rat chondrocytes. *FEBS Lett* 232:24–28, 1988
60. Burch RM, Connor JR, Axelrod J: Interleukin 1 amplifies receptor-mediated activation of phospholipase A2 in 3T3 fibroblasts. *Proc Natl Acad Sci USA* 85:6306–6309, 1988
61. Lyons-Giordano B, Davis GL, Galbraith W, Pratta MA, Amer EC: Interleukin-1 beta stimulates phospholipase A2 mRNA synthesis in rabbit articular chondrocytes. *Biochem Biophys Res Commun* 164:488–495, 1989
62. Zlotnik A, Daine B: Activation of IL 1-dependent and IL 1-independent T cell lines by calcium ionophore and phorbol ester. *J Immunol* 136:1033–1037, 1986
63. Ostrowski J, Meier KE, Stanton TH, Smith LL, Bomsztyk K: Interferon-gamma and interleukin-1 alpha induce transient translocation of protein kinase C activity to membranes in a B lymphoid cell line. Evidence for a protein kinase C-independent pathway in lymphokine-induced cytoplasmic alkalinization. *J Biol Chem* 263:13786–13790, 1988
64. Shirakawa F, Yamashita U, Chedid M, Mizel SB: Cyclic AMP—an intracellular second messenger for interleukin 1. *Proc Natl Acad Sci USA* 85:8201–8205, 1988

65. Bomsztyk K, Toivola B, Emery DW, Rooney JW, Dower SK, Rachie NA, Sibley CH: Role of cAMP in interleukin-1 induced kappa light chain gene expression in murine B cell line. *J Biol Chem* 265:9413–9417, 1990
66. Mathias S, Younes A, Kan CC, Orlow I, Joseph C, Kolisnick RN: Activation of the sphingomyelin signaling pathway in intact EL4 cells and in a cell-free system by IL-1 beta. *Science* 259:519–522, 1993
67. Kaur P, Saklatvala J: Interleukin 1 and tumour necrosis factor increase phosphorylation of fibroblast proteins. *FEBS Lett* 241:6–10, 1988
68. Kaur P, Welch WJ, Saklatvala J: Interleukin 1 and tumour necrosis factor increase phosphorylation of the small heat shock protein. Effects in fibroblasts, Hep G2 and U937 cells. *FEBS Lett* 258:269–273, 1989
69. Freshney NW, Rawlinson L, Guesdon F, Jones E, Cowley S, Hsuan J, Saklatvala J: Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. *Cell* 78:1039–1049, 1994
70. Cohen P: Dissection of the protein phosphorylation cascades involved in insulin and growth factor action. *Biochem Soc Trans* 21:555–567, 1993
71. Spriggs MK, Lioubin PJ, Slack J, Dower SK, Jonas U, Cosman D, Sims JE, Bauer J: Induction of an interleukin-1 receptor (IL-1R) on monocytic cells. *J Biol Chem* 265:22499–22505, 1990
72. Akeson AL, Mosher LB, Woods CW, Schroeder KK, Bowlin TL: Human aortic endothelial cells express the type I but not the type II receptor for interleukin 1 (IL-1). *J Cell Physiol* 153:583–588, 1992
73. Colotta F, Sironi M, Borre A, Pollicino T, Bernasconi S, Boraschi D, Mantovani A: Type II interleukin-1 receptor is not expressed in cultured endothelial cells and is not involved in endothelial cell activation. *Blood* 81:1347–1351, 1993
74. McMahan CJ, Slack JL, Mosley B, Cosman D, Lupton SD, Brunton LL, Grubin CE, Wignall JM, Jenkins NA: A novel IL-1 receptor, cloned from B cells by mammalian expression is expressed in many cell types. *EMBO J* 10:2821–2832, 1991
75. Groves RW, Sherman L, Mizutani H, Dower SK, Kupper TS: Detection of interleukin-1 receptors in human epidermis: induction of the type 2 receptor after organ culture and in psoriasis. *Am J Pathol* 145:1048–1056, 1994
76. Groves RW, Giri J, Sims J, Dower SK, Kupper TS: Inducible expression of type 2 IL-1 receptors by cultured human keratinocytes: implications for IL-1 mediated processes in epidermis. *J Immunol* 154:4065–4072, 1995
77. Ye K, Dinarello C, Clark BD: Identification of the promoter region of human interleukin 1 type I receptor gene: multiple initiation sites, high G+C content and constitutive expression. *Proc Natl Acad Sci USA* 90:2295–2299, 1993
78. Shieg JH, Peterson RHF, Moore MAS: Cytokines and dexamethasone modulation of IL-1 receptors on human neutrophils in vitro. *J Immunol* 150:3515–3524, 1993
79. Dubois CM, Ruscetti FW, Jacobsen SE, Oppenheim JJ, Keller JR: Hematopoietic growth factors upregulate the p65 type II interleukin-1 receptor on bone marrow progenitor cell in vitro. *Blood* 80:600–608, 1992
80. Symons JA, Eastgate JA, Duff GW: Purification and characterization of a novel soluble receptor for interleukin-1. *J Exp Med* 174:1251–1254, 1991
81. Alcami A, Smith GL: A soluble receptor for interleukin 1 beta encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. *Cell* 71:153–163, 1992
82. Spriggs M, Hruby DE, Maliszewski C, Pickup DJ, Sims JE, Buller RM, Van Slyke J: Vaccinia and cowpox viruses encode a novel secreted interleukin 1 binding protein. *Cell* 71:145–152, 1992
83. Cronkhite RI, Lobick JJ, Plate JMD: Heterogeneity of type-II interleukin-1 receptors. *Hum Immunol* 36:128–136, 1993
84. Heguy A, Baldari CT, Censini S, Ghiara P, Telford JL: A chimeric type II/type I interleukin-1 receptor can mediate interleukin-1 induction of gene expression in T cells. *J Biol Chem* 268:10490–10494, 1993
85. McKean DJ, Podzorski RP, Bell MP, Nilson AE, Huntoon CJ, Slack J, Dower SK, Sims J: Murine T helper cell-2 lymphocytes express type I and type II IL-1 receptors, but only the type I receptor mediates costimulatory activity. *J Immunol* 151:3500–3510, 1993
86. Curtis B, Gallis B, Overell RW, McMahan CJ, DeRoos P, Ireland R, Eisenman J, Dower SK, Sims JE: T cell interleukin 1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to interleukin 1. *Proc Natl Acad Sci USA* 86:3045–3049, 1989
87. Chiou WJ, Bonin PD, Harris PK, Carter DB, Singh JP: Platelet-derived growth factor induces interleukin-1 receptor gene expression in Balb/c 3T3 fibroblasts. *J Biol Chem* 264:21442–21445, 1989
88. Dower SK, Sims JE, Cerretti DP, Bird TA: The interleukin-1 system: receptors, ligands and signals. *Chem Immunol* 51:33–64, 1992
89. Dower SK, Qwarnstrom E, Page RC, Blanton RA, Kupper TS, Raines E, Ross R, Sims JE: Biology of the IL-1 receptor. *J Invest Dermatol* 94:68S–73S, 1990
90. Kupper TS: The role of cytokines and adhesion molecules in inflammatory skin disease. In: Oppenheim J, Gearing A (eds.). *Cytokines: Clinical Applications*. Oxford University Press, Oxford, 1993
91. Kupper TS, Ballard DW, Chua AO, McGuire JS, Flood PM, Horowitz MC, Langdon R, Lightfoot L, Gubler U: Human keratinocytes contain mRNA indistinguishable from monocyte IL-1 alpha and beta mRNA: keratinocyte "epidermal cell derived thymocyte activating factor" is identical to interleukin 1. *J Exp Med* 164:2095–2100, 1986
92. Kupper TS, Chua AO, Flood PM, McGuire J, Gubler U: Interleukin 1 gene expression in human keratinocytes is augmented by ultraviolet irradiation. *J Clin Invest* 80:430–436, 1987
93. Tron VA, Rosenthal D, Sauder DN: Epidermal interleukin-1 is increased in cutaneous T cell lymphoma. *J Invest Dermatol* 90:378–381, 1988
94. Enk AH, Angeloni VL, Udey MC, Katz SI: An essential role for Langerhans cell-derived IL-1β in the initiation of primary immune responses in skin. *J Immunol* 150:3698–3704, 1993
95. Enk AH, Katz S: Early molecular events in the induction phase of contact sensitivity. *Proc Natl Acad Sci USA* 89:1398–1402, 1992
96. Ingham E, Eady EA, Goodwin CE, Cove JH, Cunliff WJ: Pro-inflammatory levels of interleukin-1 alpha-like bioactivity are present in the majority of open comedones in acne vulgaris. *J Invest Dermatol* 98:895–901, 1992
97. Kawaguchi Y: IL-1 alpha gene expression and protein production by fibroblasts from patients with systemic sclerosis. *Clin Exp Immunol* 97:445–450, 1994
98. Cooper DA, Carr A, Vassak E, Munro V, Penny R: Immunohistological assessment of cutaneous drug hypersensitivity reactions in HIV-infected patients. *Int Conf AIDS* 10:199, 1994
99. Ferrara JL, Abhyankar S, Gilliland DG: Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. *Transplant Proc* 25:1216–1217, 1993
100. Abhyankar S, Gilliland DG, Ferrara JL: Interleukin-1 is a critical effector molecule during cytokine dysregulation in graft versus host disease to minor histocompatibility antigens. *Transplantation* 56:1518–1523, 1993
101. Sato N, Sagawa K, Sasaguri Y, Inoue O, Kato H: Immunopathology and cytokine detection in the skin lesions of patients with Kawasaki disease. *J Pediatr* 122:198–203, 1993
102. Fleming MG, Howe SF, Candel AG: Immunohistochemical localization of cytokines in nevi. *Am J Dermatopathol* 14:496–503, 1992
103. Lee WY, Fischer SM, Butler AP, Locniskar MF: Modulation of interleukin-1 alpha mRNA expression in mouse epidermis by tumor promoters and antagonists. *Mol Carcinog* 7:26–35, 1993
104. Oberyszyn TM, Sabourin CLK, Bijur GN, Oberyszyn AS, Boros LG, Robertson FM: Interleukin-1 alpha gene expression and localization of interleukin-1 alpha protein during tumor promotion. *Mol Carcinog* 7:238–248, 1993
105. Gearing A, Fincham NJ, Bird CR, Wadhwa M, Meager A, Cartwright JF, Camp RDR: Cytokines in skin lesions of psoriasis. *Cytokine* 2:68–75, 1990
106. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan L, Sauder D, Voorhees J, Fisher G: IL-1 activity is reduced in psoriatic skin. Decreased IL-1a and increased nonfunctional IL-1 beta. *J Immunol* 144:4593–4603, 1990
107. Hammerberg C, Arend W, Fisher G, Chan L, Berger A, Haskill J, Voorhees JJ, Cooper KD: Interleukin-1 receptor antagonist in normal and psoriatic epidermis. *J Clin Invest* 90:571–583, 1992
108. Williams IR, Kupper TS: Epidermal expression of ICAM-1 is not a primary inducer of cutaneous inflammation in transgenic mice. *Proc Natl Acad Sci USA* 91:9710–9714, 1994
109. Williams IR, Ort RJ, Kupper TS: Keratinocyte expression of B7-1 in transgenic mice amplifies the primary immune response to cutaneous antigens. *Proc Natl Acad Sci USA* 91:12780–12784, 1994
110. Nakamura K, Williams IR, Kupper TS: Overexpression of keratinocyte derived monocyte chemoattractant protein 1 (MCP-1) in transgenic mice causes accumulation and redistribution of cutaneous dendritic and Langerhans cells. (submitted)