

# **Skin Immune Mechanisms in Health and Disease**

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## PART I: Immune Components of Skin

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# 2

## Skin Immune System: Humoral

The humoral component of the skin immune system consists of various factors including complement components, prostaglandins, leukotrienes, secretory immunoglobulins, cytokines, chemokines, neuropeptides, and anti-microbial peptides (Table 2.1). A detailed description of anti-microbial peptides will be given in Chapter 3.

### 2.1 Complement components

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The complement system participates in both the innate (see Chapter 3) and acquired immune responses to pathogens and other foreign antigens. The epidermis contains the complement components necessary for generation of the initial C3 convertase of the alternative pathway. In addition KCs synthesize complement regulatory proteins, which allow them to modulate complement activation and protect the epidermis from damage.

#### 2.1.1 C3 and B

KCs produce two components of the alternative complement pathway, C3 and B [1] (Fig. 2.1).

**C3** is a central component of the alternative and classical complement pathways and is composed of two polypeptides linked by disulphide bonds. In the alternative complement pathway, a spontaneously formed C3 convertase cleaves C3 to give C3b and the anaphylactic C3a fragment. C3b has an intramolecular thioester bond, which allows it to bind to nearby cell surface molecules and immune complexes, and multiple binding sites for complement components. Binding of factor B, factor D and properdin (factor P) to cell-bound C3b results in the formation of stable C3 convertases (C3bBb), which can cleave C3 to generate more C3b.

**Factor B** is a single chain polypeptide that, on binding to C3b, is cleaved by factor D. The smaller Ba fragment is released, whilst the second fragment Bb, which contain domains with homology to the catalytic chains of other serine proteases, remains associated with C3b.

In this way, huge numbers of C3b molecules are generated which coat the surface of the bacterial cell and act as opsonins for uptake by phagocytes expressing complement receptors CR1, CR2, CR3 and CR4, or trigger activation of the terminal pathway of complement resulting in assembly of the membrane attack complex and eventual cell lysis.

Synthesis of C3 and B by cultured KCs is constitutive, and can be increased further by various proinflammatory cytokines, particularly TNF- and IFN- , respectively [1]. Moreover, KC-derived C3 is regarded as a potential source of C3d,g, a constituent of the sublamina densa region of normal epidermal basement membrane. C3d,g is formed from C3b as a result of two cleavage steps by

**Table 2.1** Humoral components of the SIS

Components	Epidermal producer cell types	Dermal producer cell types
<b>Complement system</b>		
C3	KCs	None
B	KCs	None
CR1	KCs	None
Membrane cofactor protein	Intercellular spaces, particularly of basal layers	Endothelial
Decay accelerating factor	Basement membrane	Elastic fibres
CD59	KCs	None
<b>Polyunsaturated fatty acid metabolites</b>		
13-HODE	Lamellae in stratum corneum	None
PGE <sub>2</sub> , PGF <sub>2</sub> , PGD <sub>2</sub>	KCs	Fibroblast - PGE <sub>2</sub>
15-HETE	KCs	None
LTB <sub>4</sub>	KCs, converted from PMN-derived LT-A <sub>4</sub>	None
<b>Secretory Immunoglobulins</b>		
Secretory component	Basement membrane, KC surface, sweat glands	None
IgA	None	Sebaceous and eccrine sweat glands
<b>Cytokines and Chemokines</b>		
Proinflammatory, immunomodulatory cytokines, growth factors, CSFs and/or interferons	KCs and LCs	Endothelial, T, mast, dendritic, macrophage, fibroblast, sebaceous gland
CXCL and CC chemokines	KCs and LCs	Endothelial, T, mast, dendritic, macrophage, fibroblast
<b>Neuropeptides</b>		
Substance P	Sensory neurons	Sympathetic nerves
CGRP	Sensory nerve endings close to LCs	Sensory nerves surrounding blood vessels, in sweat glands
Neuropeptide Y	Nerve fibres in basal layer, (LCs in atopic dermatitis)	Around blood vessels, sweat glands

Components	Epidermal producer cell types	Dermal producer cell types
Somatostatin	KCs, on LC membrane	Dendritic cell subset, Meissner corpuscles, mast cells
VIP	None	Sweat and apocrine glands, arterial blood vessels, hair follicles, mast cells
PACAP	None	Close to E/D junction, sweat glands, blood vessels, hair follicles
POMC-derived peptides	KCs, LCs and melanocytes	Endothelial, fibroblast
<b>Protease-activated receptors</b>		
PAR-1	KCs	Endothelial, fibroblast, vascular smooth muscle
PAR-2	KCs, highest in granular layer	Endothelial, hair follicles, sweat glands, dendritic
<b>Vanilloid receptors</b>		
Vanilloid receptor-1	KCs, sensory nerves	Sweat and sebaceous glands, blood vessels, hair follicles, mast cells

factor I in combination with various cofactors (see below), with the release of fragments C3f and C3c. In various inflammatory skin diseases a greater C3d,g reactivity is observed and is typically associated with C3c deposition.

### 2.1.2 Complement regulatory proteins

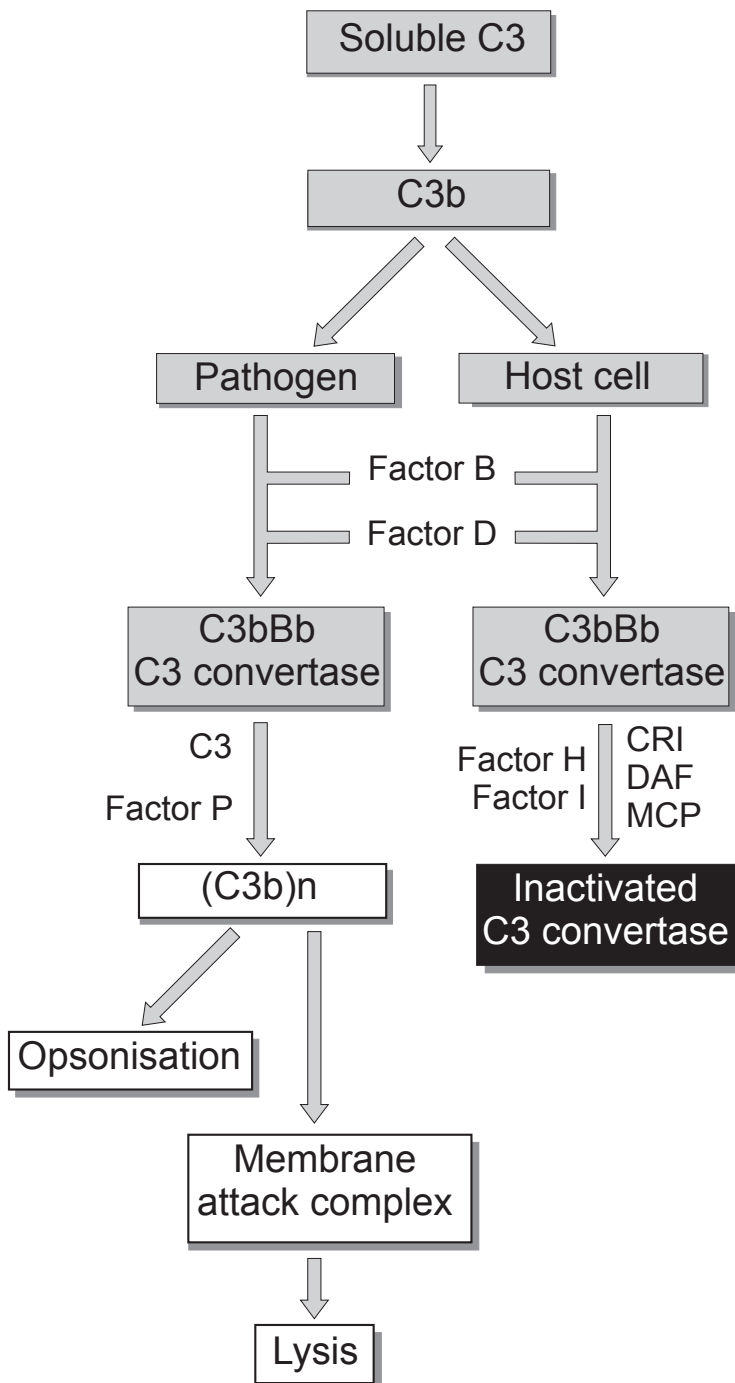
Four complement regulatory proteins, CR1, MCP, DAF and CD59, whose role is to protect KCs from damage induced by complement activation, are present in normal skin [2] (Fig 2.1).

**CR1** (CD35) is a highly polymorphic, single-chain membrane protein that binds C3b via sites in its repeat domains, and has cofactor and delay accelerating activity. It is expressed by nearly all human peripheral blood cells and by KCs in the skin.

**MCP** (Membrane cofactor protein; CD46) is a membrane-bound complement regulatory protein that has cofactor activity for factor I, a serine protease which degrades C3b. It is composed of four short consensus repeats, a sequence of serine, threonine and proline residues, and a transmembrane region. MCP is located in the intercellular spaces of epidermis, being higher in the basal layers than in the granular layer, and on endothelial cells in the dermis.

**DAF** (Decay accelerating factor; CD55) is a membrane-bound complement regulatory protein whose function is to regulate the formation of C3 convertases and to decrease their stability.

**Figure 2.1** The alternative complement pathway. CR1 = complement receptor 1; DAF = decay-accelerating factor; MCP = membrane cofactor of proteolysis.



DAF, which has a similar structure to MCP, is attached to the epidermal basement membrane by a glycosylphosphatidylinositol (GPI) anchor; the protein is also found on elastic fibres in the dermis.

**CD59** is a membrane-associated complement inhibitory protein, which is constitutively produced by KCs *in vitro*. Activation of CD59 expressed by KCs by its T cell ligand CD2 results in the production of the cytokines IL-1, IL-6 and GM-CSF.

UVB light up-regulates the levels of MCP, DAF and CD59, but has no effect on C3 and factor B production. In contrast, various cytokines have no effect on expression of the complement regulatory proteins with the exception of TGF- $\beta_1$ ,  $\beta_2$  and  $\beta_3$ , which, together with unknown additional factors, enhance MCP and CD59 levels.

In addition to acting as complement regulatory proteins, these membrane proteins are also used by microorganisms to gain entry into host cells. Thus CR2 on T and B cells has been shown to act as a receptor for Epstein Barr virus, and MCP and DAF act as receptors for the measles virus and echovirus. MCP expressed by KCs is also a receptor for the M proteins of group A streptococci.

## 2.2 Polyunsaturated fatty acid metabolites

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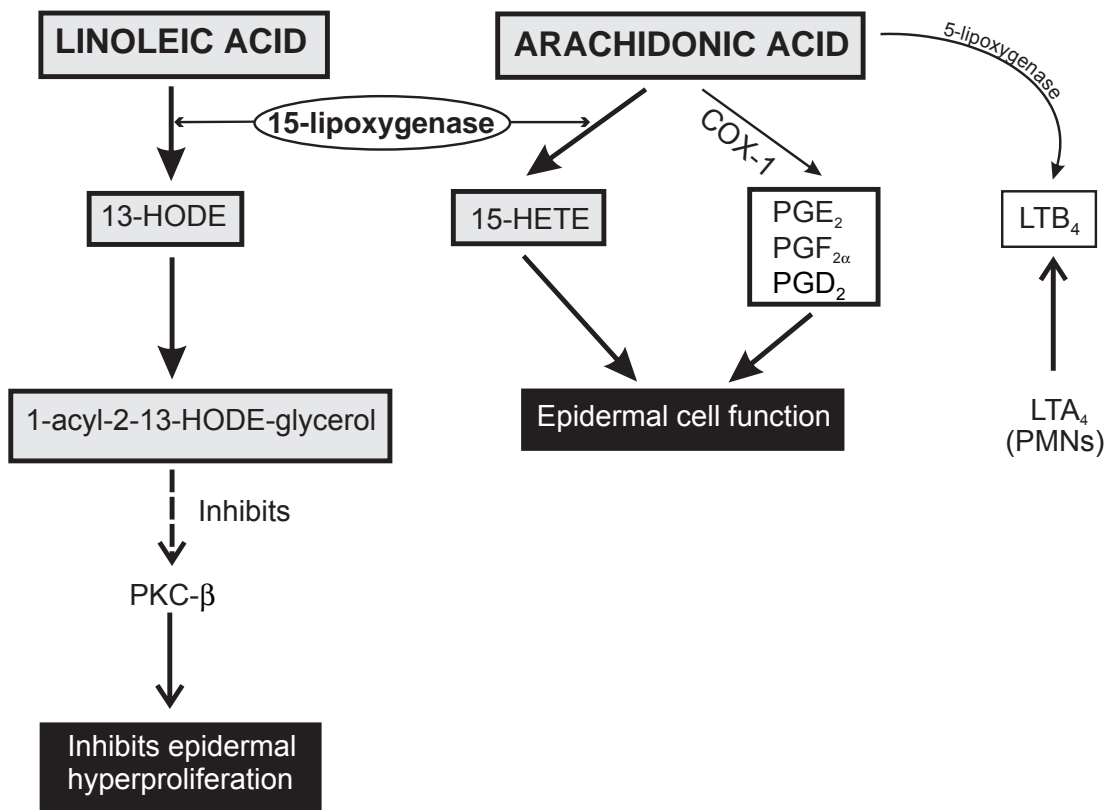
### 2.2.1 Linoleic acid

The ability of the epidermis to act as a barrier to prevent water loss is attributed to the lipid bilayers or lamellae present in the intercellular spaces of the stratum corneum. The most abundant polyunsaturated fatty acid in human skin, which plays an essential role in the maintenance of this epidermal water barrier, is the 18-carbon polyunsaturated fatty acid, linoleic acid. However, deficiency of linoleic acid not only results in excessive water loss, but also in a characteristic scaly skin disorder implicating the fatty acid in regulation of epidermal proliferation. Epidermis contains the enzyme 15-lipoxygenase, which metabolises linoleic acid to 13-hydroxyoctadecadienoic acid (13-HODE), the major metabolite, and small amounts of 9-HODE [3] (Fig. 2.2). Normal skin epidermis is unique in that it preferentially metabolises linoleic acid to 13-HODE, as it is unable to transform it to  $\gamma$ -linolenic acid. 13-HODE is then substituted into diacylglycerol to give 1-acyl-2-13-HODE-glycerol, which selectively suppresses the  $\alpha$  isoform of membrane-associated epidermal protein kinase C resulting in inhibition of epidermal hyperproliferation.

### 2.2.2 Arachidonic acid

The second most prominent polyunsaturated fatty acid in the skin is the 20-carbon fatty acid arachidonic acid, which comprises approximately 9% of the total fatty acids in epidermal phospholipids. Arachidonic acid is released from membrane phospholipids by the action of cytosolic phospholipase A2 present in the upper epidermal layers. This enzyme, which is induced by UV light, chemicals and other factors that injure the skin, is associated with various normal skin functions, such as cell proliferation and differentiation, wound healing and host defence against bacteria. Two different pathways can convert the liberated arachidonic acid to either prostaglandins or 15-HETE (15-hydroxyeicosatetraenoic acid) [3] (Fig. 2.2).

**Figure 2.2** Metabolism of linoleic acid and arachidonic acid in the epidermis of skin. 13-HODE = 13-hydroxyoctadecadienoic acid; PKC- $\beta$  =  $\beta$  isoform of protein kinase C; 15-HETE = 15-hydroxyeicosatetraenoic acid; COX-1 = cyclooxygenase-1; PG ( $E_2$ ,  $F_{2\alpha}$ ,  $D_2$ ) = prostaglandin ( $E_2$ ,  $F_{2\alpha}$ ,  $D_2$ );  $LTB_4/A_4$  = leukotriene  $B_4/A_4$ .



### 2.2.2.1 Prostaglandins

The first pathway converts arachidonic acid by enzymatic peroxidation and cyclooxygenation to the prostaglandins PGE<sub>2</sub>, PGF<sub>2</sub> and PGD<sub>2</sub>. Two isoforms of prostaglandin H synthase, also known as cyclooxygenase (COX), enzymes are involved in the pathway; COX-1 (constitutive) which maintains basal levels of prostaglandins necessary to maintain physiological epithelial function, and COX-2 (inducible) which is induced by proinflammatory stimuli and UV light, resulting in the production of high levels of the lipid mediators. COX-1 is constitutively expressed in KCs throughout the normal epidermis, in endothelial cells of small blood vessels, and in sweat gland epithelium. In contrast COX-2 expression is normally very low and restricted to a small number of suprabasal KCs in the epidermis. Altered levels of COX-2 are a characteristic feature of epithelial tumours including squamous cell carcinoma of the skin.

PGE<sub>2</sub> plays an important role in the skin, not only in homeostasis, but also in repair of the epidermis after wounding when levels are increased *in vivo* [4]. Furthermore, there is increased PGE<sub>2</sub> synthesis associated with high mitotic activity in non-confluent proliferating KCs in culture. These effects are mediated via specific G protein-coupled PGE receptors, of which there are 4 different subtypes, two of which are expressed in the epidermis by KCs and LCs [5].

### 2.2.2.2 15-HETE and Leukotrienes

The second pathway generates 15-hydroxyeicosatetraenoic acid (15-HETE), a mediator of basal epithelial function, from arachidonic acid. This process is catalysed by the 15-lipoxygenase enzyme, which has high activity in the epidermis. In contrast epidermal 5-lipoxygenase, which catalyses the conversion of arachidonic acid to the ether-linked phospholipid, leukotriene LTB<sub>4</sub>, is normally present at only very low levels in KCs, although the enzyme can be up-regulated by culture of KCs under conditions that induce differentiation. However, levels of LTB<sub>4</sub> may be elevated in KCs due to its conversion from LTA<sub>4</sub> derived from activated leukocytes such as PMNs. This involves the enzyme LTA<sub>4</sub> hydrolase present in both basal and suprabasal KCs, which is subsequently inactivated. This process of transcellular LTB<sub>4</sub> synthesis may account, at least in part, for the elevated levels of this proinflammatory leukotriene in skin lesions of psoriasis. In addition to being a potent chemotactic factor for T lymphocytes and PMNs, LTB<sub>4</sub> exerts effects on vascular permeability and blood flow, and can stimulate DNA synthesis in cultured KCs.

## 2.3 Secretory immunoglobulins

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The polymeric immunoglobulin receptor, secretory component has been detected in normal skin, either along the epidermal basement membrane or focally on the surface of KCs, and in sweat glands by immunostaining [6]. This suggests that KCs may be able to interact with IgA, which could be of relevance to certain skin diseases such as dermatitis herpetiformis in which IgA is deposited underneath the epidermal basement membrane.

Furthermore, immunostaining for IgA has been found in sebaceous glands and in various parts of the eccrine sweat glands in normal skin [7]. IgA was concentrated near the pilosebaceous duct opening in sebaceous glands, and all over the sweat ducts, whilst only scattered IgA-positive cells were seen in the secretory parts of the sweat gland. Immunoelectron microscopy further suggested that IgA was taken up by endocytosis into glandular cells and processed. These findings suggest that IgA present in sebum and sweat may play a role in the inactivation of invading microorganisms as part of the skin's defence mechanisms.

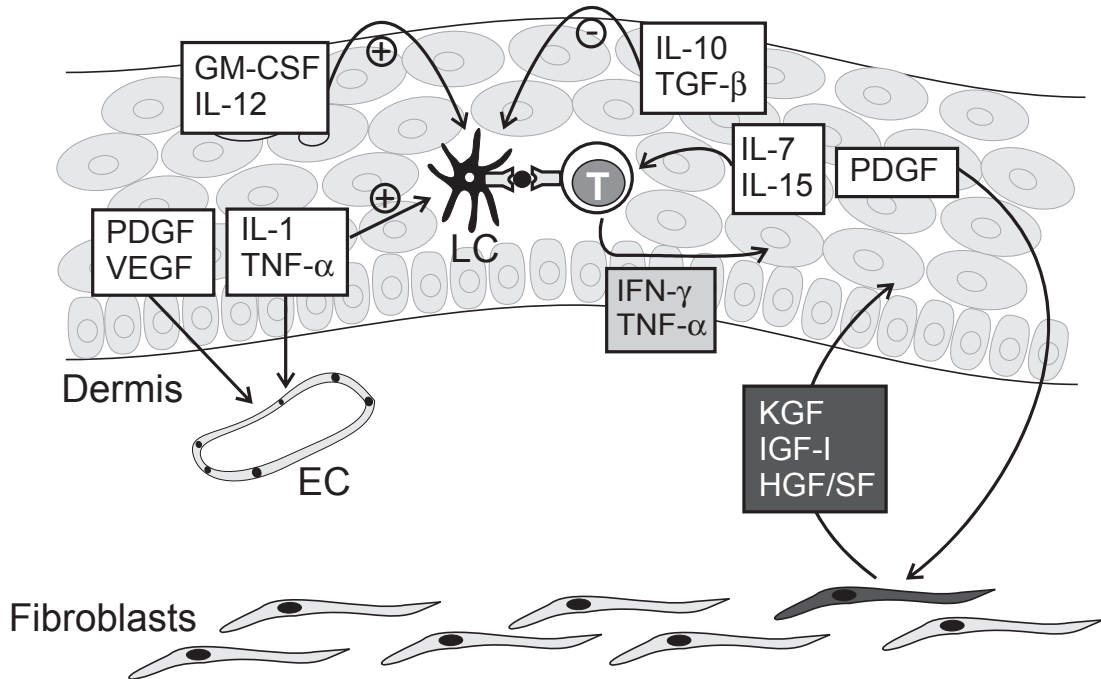
## 2.4 Cytokines and Chemokines

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As discussed in Chapter 1, KCs are the major source of cytokines and chemokines in skin (summarised in Table 1.3). Many of these cytokines are constitutively expressed at low levels and are up-regulated in response to a variety of stimuli such as trauma, bacterial infections, UV irradiation and chemical irritants. The cytokines produced by KCs can be subdivided into various subclasses: proinflammatory, immunomodulatory, growth factors, colony stimulating factors and

interferons [8].

KC-derived cytokines can play autocrine and/or paracrine roles in the skin. In addition to activating the proliferation and differentiation of the cells that produce them, the main function of these cytokines is to regulate the function of other cell types such as LC and T cells in the epidermis, and endothelial cells and fibroblasts in the dermis (Fig. 2.3). These other skin cell types also produce their own repertoire of cytokines, which can, in turn, affect KC function (see Chapter 1). Furthermore, chemokines produced by KCs attract leukocytes into the epidermis from the circulation.



**Figure 2.3** Cytokine-mediated interaction between KCs and other skin cells. KCs produce cytokines that have either a stimulatory (+) or inhibitory (-) effect on antigen presentation by LCs to T cells. T cells and fibroblasts also produce cytokines that affect KC function and growth. EC = endothelial cell.

### 2.4.1 Interaction between keratinocytes and Langerhans cells

LC function is regulated by various cytokines produced by KCs, particularly GM-CSF which is essential for LC viability and up-regulates costimulatory molecules B7-1 and B7-2 on the cell surface *in vitro*. Furthermore, the induction of dendritic cells from precursors isolated from the blood and bone-marrow has an absolute requirement for GM-CSF *in vitro*. In addition, IL-1 potentiates the antigen-presenting function of LCs, and IL-12 promotes their accessory cell function, augmenting IFN- production in responder T cells.

In contrast, the inhibitory cytokine IL-10 inhibits ICAM-1 expression on LCs thus decreasing their interaction with LFA-1-positive T cells. In addition, IL-10 prevents the up-regulation of CD80 on LCs, leading to inhibition of Th-1 cell responses. TGF- $\beta_1$  has also been shown to be inhibitory, at least in mice, for MHC Class II up-regulation on LCs by cytokines such as IL-1, TNF- $\alpha$  and GM-CSF. TGF- $\beta_1$  is also involved in the synthesis of Birbeck granules in dendritic cells induced in culture by GM-CSF and IL-4.

IL-1 and TNF- $\alpha$  produced by KCs play a key role in the emigration of LCs from the epidermis to lymph nodes. This is discussed in detail in Chapter 4.

### 2.4.2 Interaction between keratinocytes and T cells

KCs produce two cytokines that promote T cell growth and activity, IL-7 and IL-15, which bind to the  $\alpha$  and/or  $\beta$  chains of the IL-2 receptor. IL-7 induces cytokine production and expression of the IL-2  $\beta$ -chain (CD25) receptor by T lymphocytes, and has been implicated in trafficking of T cells into the epidermis via its effects on their adhesion to laminin-5, a constituent of the basement membrane. IL-15, in common with IL-2, stimulates T cell proliferation and activates NK cells.

Conversely, IFN- $\gamma$  produced by activated T cells in inflammatory skin stimulates KCs via specific receptors on their surface inducing HLA-DR and ICAM-1 expression. ICAM-1 can also be induced on KCs, to a lesser degree, by TNF- $\alpha$ . This allows interaction between T cells and KCs via LFA-1 and ICAM-1, respectively, mediating T cell infiltration into the epidermis.

### 2.4.3 Interaction between keratinocytes and endothelial cells

IL-1 and TNF- $\alpha$  produced by KCs induce various effects on dermal endothelial cell function, including the production of various cytokines (including IL-1 and IL-6) and of PGI $_2$ , NO and PAF, and the up-regulation of ICAM- and VCAM-1 expression on the endothelial cell surface. The increased expression of adhesion molecules, and the vasodilatory effects of PGI $_2$  and NO facilitate the migration of T cells from the circulation into the skin during inflammation.

In addition, KCs synthesize VEGF, which induces proliferation of dermal endothelial cells via the two VEGF receptors, kdr and flt-1. Levels of VEGF are induced by hepatocyte growth factor/scatter factor (HGF/SF) produced by fibroblasts and smooth muscle cells, and by cytokines such as KC-derived TGF- $\beta$  and EGF.

Homodimers and heterodimers of the A and B isoforms of PDGF synthesized by KCs stimulate proliferation of microvascular endothelial cells, and other dermal cells (see below).

### 2.4.4 Interaction between keratinocytes and fibroblasts

KC-derived PDGF stimulates proliferation of dermal cells such as fibroblasts, smooth muscle cells (and microvascular endothelial cells) and acts as a chemoattractant for fibroblasts, and PMNs, monocytes and smooth muscle cells, during wound healing. In turn, fibroblasts release growth factors for KCs, in particular KGF that exerts potent mitogenic effects, and also IGF-I, which regulates KC proliferation and differentiation in combination with EGF and EGF-like factors. Fibroblasts also synthesize HGF/SF, which induces VEGF production by KCs.

## 2.4.5 Leukocytes attracted by keratinocyte-derived chemokines

KCs are capable of producing a range of chemokines belonging to the CXC and CC subfamilies, which can attract various types of leukocytes into the epidermis [8].

Basal KCs constitutively synthesize CCL27 (CTACK) and CCL20 (MIP-3), chemokines responsible for the trafficking of T cells and LCs, respectively, into the epidermis of both non-inflamed and inflamed skin [9,10]. CCL27 attracts T cells bearing CCR10, a CCL27 receptor, towards the epidermis along a chemotactic gradient and, during inflammation when CCL27 is up-regulated, some of these T cells enter the epidermis. On the other hand, CCL20 is largely responsible for recruitment of CCR6 receptor-positive LCs into the epidermis, whilst a lesser role is played by another chemokine, CXCL12 (SDF-1), a weak chemoattractant produced by basal KCs that is recognised by LCs via their CXCR4 receptors.

In inflammatory skin diseases such as psoriasis, IFN- $\gamma$  produced by Th-1 cells induces KCs to produce CXCL9 (Mig) and CXCL10 (IP-10) which are chemoattractants for CXCR3-positive activated Th-1 cells. IFN- $\gamma$  and TNF- $\alpha$  also induce CCL5 (RANTES) and CCL2 (MCP-1) production by KCs which can attract not only activated T cells, but also monocytes, dendritic cells, NK cells, and in the case of CCL5, eosinophils providing that they express the corresponding receptors (CCR1, CCR3 or CCR5 for CCL5, and CCR2 for CCL2). PMNs expressing CXCR1 or CXCR2 receptors are also present in the epidermis of psoriatic skin lesions, drawn in from the circulation by increased levels (up-regulated by TNF- $\alpha$ ) of epidermal CXCL1 (GRO- $\alpha$ ) and CXCL8 (IL-8).

## 2.4.6 Cytokine inhibitors

The production or functions of cytokines in the skin are regulated in various ways. In the case of IL-1, inhibition of the cytokine can occur by binding to the IL-1RII receptor, which has no cytoplasmic domain and cannot therefore transduce a signal to the inside of the cell, or by binding to the soluble forms of the extracellular domains of the receptors released from the surface of the cell. In addition, IL-1ra acts as a competitive inhibitor of IL-1 by binding to IL-1RI without subsequent signal transduction.

Cytokines can also suppress the production of other cytokines, a good example of which is the reciprocal relationship between IFN- $\gamma$  and IL-4. Thus IFN- $\gamma$  inhibits the development of Th-2 cells producing IL-4, whilst conversely, IL-4 inhibits the development of Th-1 cells producing IFN- $\gamma$ . Furthermore, the cytokine IL-10, also called cytokine synthesis and inhibitory factor, inhibits the synthesis of various cytokines, including IFN- $\gamma$  by Th-1 cells.

Another mechanism by which the effects of cytokines are regulated is by inhibition of components of the cytokine-signalling pathway. Inhibition of signal transducers and activators of transcription (STAT) phosphorylation is mediated in KCs by two members of the SOCS/CIS family, SOCS-1 and SOCS-3, which are induced by cytokine or UVB stimulation of KCs [11]. SOCS-1 is induced by IFN- $\gamma$  or IL-4 and is a crucial inhibitor of IFN- $\gamma$ /STAT-1 and IL-4/STAT-6 signalling in KCs. SOCS-3 is induced by IFN- $\gamma$  or IL-6, and regulates IFN- $\gamma$ /STAT-1 and IL-6/STAT-3 signalling.

## 2.5 Neuropeptides

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Neuropeptides are released from sensory or autonomic nerve fibres in the skin in response to stimuli such as physical, chemical or thermal injury, or UV irradiation. In addition to their well-defined functions within neurons, neuropeptides modulate the function of immunocompetent and inflammatory cells, and cutaneous cells through high affinity neuropeptide receptors or by direct activation of intracellular signalling pathways. These effects are terminated by degradation of neuropeptides by specific endopeptidases such as neutral endopeptidase, a member of a family of cell surface zinc metalloproteinases present in skin and other tissues. Furthermore, immunocompetent cells, and several epidermal as well as dermal cells can themselves synthesize a variety of neuropeptides including substance P, calcitonin gene-related peptide (CGRP), somatostatin, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP) and proopiomelanocortin (POMC)-derived peptides (Table 2.2)[12,13].

### 2.5.1 Substance P

Substance P is a member of the tachykinin peptide family, which also includes neurokinin A (NKA), neurokinin B (NKB), neuropeptide K and neuropeptide  $\delta$ , and is derived from precursors  $\delta$ -preprotachykinin and  $\delta$ -preprotachykinin. NKA is also derived from the latter and shares common biological activities with substance P. There are three tachykinin (neurokinin) G-protein coupled receptors (NK1R, NK2R and NK3R), which preferentially bind substance P, NKA and NKB, respectively. In the skin, KCs, endothelial cells, mast cells, fibroblasts, Merkel cells and LCs all express tachykinin receptors.

#### 2.5.1.1. Cutaneous neuroinflammation

Substance P is a potent vasodilator acting directly on vascular smooth muscle and enhancing NO production by endothelium. It also acts as an IgE-independent stimulator of histamine release from mast cells. These two actions may explain the cutaneous weal and flare response, respectively, in response to antidromic (in the opposite direction to normal) stimulation of sensory nerves. NKA is less potent than substance P in inducing flare and itch in human skin, which may be due to a poorer ability to stimulate histamine release.

Substance P can also modulate inflammation via its effects on proliferation of various skin cell types. Thus substance P, and also NKA, can stimulate the growth of KCs, fibroblasts, endothelial cells and arterial smooth muscle cells *in vitro*, although suppression of KC proliferation has also been demonstrated under certain culture conditions. Furthermore, substance P and NKA can directly induce NGF in KC. This may be relevant in the maintenance and regeneration of cutaneous nerve fibres in both normal skin and in inflammatory reactions and wound healing.

#### 2.5.1.2 Modulation of immune cell function

Substance P, in common with other neuropeptides, modulates various immune and inflammatory functions such as the production of proinflammatory and immunomodulatory cytokines by various cell types including KCs, T and B cell proliferation, NK and mast cell activity, and expression

**Table 2.2** Neuropeptides and their receptors

NP	NPR	Stimulatory effects	Inhibitory effects
Substance P	NKR1	Potent vasodilator IgE-independent mast cell histamine release Growth of epidermal and dermal cells KC cytokines and NGF production Mast cell activity ICAM-1, VCAM-1 on endothelial cells Promotes induction and elicitation of CHS to haptens	
CGRP	CLR (+ RAMP-1)	Vasodilator Induces hapten tolerance Melanocyte proliferation <i>Endothelial cells</i> : proliferation, IL-8 production and adhesion molecule expression	LC antigen-presentation (incr. IL-10, decr. B7-2) DTH and CHS induction
Neuropeptide Y	Y2R	Vasoconstrictor Sweat regulation?	Sweat regulation?
Somatostatin (14 AA)	SR-5 subtypes	Fibroblast proliferation	
VIP	VIPR	Vasodilator Induces vascular permeability IgE-independent mast cell histamine release KC proliferation Leukocyte chemotaxis, at low doses Increases B cell IgM production	DTH PLA <sub>2</sub> Thymocyte and mitogen-induced PBL proliferation PBMN and PMN migration, at high doses B cell IgA production NK activity ACD
PACAP-38	VPAC1 VPAC2	IgE-independent mast cell histamine release	CHS induction DTH elicitation LC antigen presentation (incr. IL-10, decr. B7-2)

NP	NPR	Stimulatory effects	Inhibitory effects
POMC-derived peptides	MC-1R (MC-5R)	Upregulates IL-10 Hapten tolerance B cell IgE production IL-1-induced endothelial IL-8 production Proliferation and melanin production by follicular melanocytes	Cytokine production by various cell types Macrophage NO production CHS B cell IgE production, at high doses Mast cell histamine release IL-1 -induced KC CXCL1 and CXCL8 expression LPS-induced endothelial adhesion molecule expression Monocyte and dendritic cell Class I, B7-2, CD40, ICAM-1
Protease-activated receptors	Protease-activated receptor-1	<i>KCs and fibroblasts:</i> Ca <sup>2+</sup> influx, proliferation, IL-6 and/or GM-CSF production <i>Endothelial cells:</i> Ca <sup>2+</sup> influx, von Willebrand factor, P-selectin, E-selectin, ICAM-1, VCAM-1, NO, PDGF, ET-1, VEGFR expression, proliferation, cell contraction, permeability	KC differentiation
	Protease-activated receptor-2	<i>KCs:</i> Ca <sup>2+</sup> influx, IL-6, IL-8, GM-CSF, phagocytosis, melanosome uptake <i>Endothelial cells:</i> Ca <sup>2+</sup> , IL-6, IL-8, NF- B	<i>KCs:</i> proliferation, differentiation, TG-1 and involucrin expression
	Vanilloid receptor-1	<i>KCs:</i> Increases CXCL8, PGE <sub>2</sub> and COX-2	

of adhesion molecules ICAM-1 and VCAM-1 by endothelial cells. Furthermore, substance P has been shown to promote the induction and elicitation of contact hypersensitivity to haptens in skin (see Chapter 4). The latter was demonstrated in mice by genetic deletion of substance P-degrading neutral endopeptidase, which is normally present in KCs, dermal microvascular endothelial cells and hair follicles [14]. This resulted in a markedly augmented ear swelling response, probably due to the accumulation of proinflammatory substance P. In contrast, deletion of NK1R inhibited the proinflammatory effects of substance P, markedly reducing the contact hypersensitivity response [15]. These studies illustrate the involvement of the cutaneous nervous system in the inflammatory

response to contact allergens.

Cytokines such as IL-1 enhance sympathetic neurone-derived substance P production, which has been detected by immunostaining in nerve fibres in lesional skin of patients with atopic dermatitis, psoriasis and other inflammatory skin diseases. In patients with atopic dermatitis, substance P regulates the proliferation and cytokine production of PBMC in response to house dust mite allergens [16].

## 2.5.2 Calcitonin gene-related peptide

The calcitonin peptide family consists of five members; calcitonin, amylin, adrenomedullin and two calcitonin gene-related peptides CGRP- or CGRP-1 and CGRP- or CGRP-2. The receptor for CGRP consists of the calcitonin-like receptor (CRLR) expressed with a receptor-activity modifying protein (RAMP)-1. (CRLR expressed with RAMP2/3 is specific for adrenomedullin). CGRP receptors are expressed on various inflammatory cell types including macrophages, mast cells and PMNs, and in the skin, on KCs, melanocytes, LCs and dermal microvascular endothelial cells.

### 2.5.2.1 Cutaneous neuroinflammation

CGRP released from efferent nerve fibres contributes to neuroinflammation by inducing vasodilation and plasma extravasation. However, unlike substance P, VIP and somatostatin, CGRP will only cause a weak weal and flare reaction and only at relatively high concentrations. Thus intradermally injected CGRP induces a gradually developing, but long-lasting and strong erythematous response that is not dependent upon histamine release by mast cells.

### 2.5.2.2 Modulation of immune cell function

Unmyelinated afferent sensory nerve fibres containing CGRP in the skin have been detected in association with either substance P or, more commonly, somatostatin (see below), in free epidermal nerve endings and in axons surrounding blood vessels in the dermis and dermal papillae. CGRP-positive sensory axons containing weak somatostatin-specific staining have also been detected in sweat glands. In the epidermis, CGRP-containing nerves have been shown in close association with LCs and, furthermore, CGRP has been observed on the surface of some of these cells. These findings are relevant in view of the inhibitory effects of the neuropeptide on the antigen-presenting function of LCs [17]. The exact mechanism is unknown but is believed to be via induction of IL-10 release by LCs, and down-regulation of costimulatory molecules such as B7-2 on the surface. In addition, CGRP inhibits the induction of delayed-type and contact hypersensitivity, and induces hapten-specific tolerance via the induction of IL-10 in animal models [18,19]. CGRP is up-regulated in skin after UV irradiation suggesting a role for this neuropeptide in UV-induced immunosuppression. CGRP has also been shown to stimulate melanocyte proliferation accompanied by accumulation of intracellular cAMP suggesting that the nervous system may be involved in the regulation of pigmentation of the skin [20].

In the dermis, CGRP stimulates proliferation and IL-8 production by endothelial cells and up-regulates their expression of adhesion molecules. These effects may be relevant in psoriasis and other inflammatory skin diseases such as vitiligo in which CGRP levels are up-regulated. Other

immunomodulatory effects mediated by CGRP include the inhibition of mitogen/antigen-induced peripheral blood mononuclear cell proliferation and cytokine production, and the stimulation of T cell chemotaxis.

### 2.5.3 Neuropeptide Y

Neuropeptide Y is found in nerve fibres located around the cutaneous vasculature, and plays a role in regulation of skin blood flow via vasoconstrictive effects mediated by Y2 receptors. Neuropeptide Y is not only present in the wall of arteries, arterioles and veins, but also in nerve fibres in the basal layer of the epidermis, and in association with eccrine sweat glands (and to a lesser degree apocrine and sebaceous glands and hair follicles) suggesting a role in the regulation of sweat production. In atopic dermatitis, neuropeptide Y-like immunoreactivity has been found on LCs in the epidermis.

### 2.5.4. Somatostatin

Somatostatin (somatotropin release-inhibiting factor) occurs in two biologically active forms, consisting of either 14 or 28 amino acids. It is a ubiquitous peptide with inhibitory effects on several neuropeptides and hormones, and which possesses immunomodulatory properties, which has led to its use as a treatment for psoriasis.

In the skin, the 14 amino acid somatostatin is found in the epidermis and on the LC membrane. In addition, a subset of DDC is somatostatin-positive, and the neuropeptide is associated with Meissner corpuscles in the dermal papillae. Somatostatin, which is also produced by mast cells and PMNs, mediates its effects either indirectly through other molecules, or directly via receptors, of which there are 5 subtypes, on target cells. Blood vessels, smooth muscle, sweat glands and fibroblasts in the dermis express somatostatin receptors. Fibroblasts, which express subtype 2/3 receptors, are induced by somatostatin to proliferate but the effect is weak.

### 2.5.5 VIP

VIP and peptide histidine methionine (PHM) are two members of the glucagon-secretin family, which play a role in skin. Nerves secreting these peptides are found around the glandular cells, ducts and myoepithelial cells of the eccrine sweat glands, where they stimulate sweat production, around the arterial section of the superficial and deep vascular plexuses, and adjacent to hair follicles. PHM-secreting nerve fibres are also seen close to apocrine glands. Nerve fibres secreting VIP have been observed in psoriasis, atopic dermatitis and other inflammatory skin diseases.

VIP induces vasodilation, increases vascular permeability and regulates blood flow, and when released from sensory nerve endings or mast cells, can induce KC proliferation via specific VIP receptors by stimulating adenylate cyclase activity. In common with substance P, VIP induces histamine release by mast cells in an IgE-independent manner, but in contrast to that neuropeptide, VIP exerts anti-inflammatory effects, such as the suppression of experimental delayed hypersensitivity reactions [21] and inhibition of phospholipase A2. VIP is also inhibitory in its effects on various immune cell functions such as proliferation of thymocytes and of mitogen-induced peripheral blood lymphocytes, but preferentially induces chemotaxis of T lymphocytes. In addition, VIP decreases IgA and stimulates IgM production by B cells, and inhibits natural killer cell activity. Interestingly, VIP

exerts an inhibitory effect on established allergic contact dermatitis, possibly in part by stimulating IFN- production by peripheral blood T cells.

### 2.5.6 PACAP

PACAP is a neuropeptide homologous with VIP, which occurs as two variants, PACAP-27 and C-terminally extended PACAP-38. In the skin, PACAP-38 is located predominantly in dermal nerve fibres close to the dermal-epidermal border, hair follicles, blood vessels and sweat glands, whilst the PACAP-27 variant is absent. The levels of PACAP-38 are elevated in psoriasis, as compared to those of normal skin, suggesting its involvement in the disease process. High affinity receptors for PACAP (VPAC1 and VPAC2) are present in skin on eccrine sweat and apocrine glands in the dermis and on LCs in the epidermis.

PACAP is an immunosuppressive neuropeptide which prevents induction of contact hypersensitivity, and inhibits the ability of LCs to elicit delayed hypersensitivity in previously immunized mice or present antigen to antigen-specific T cells *in vitro*, probably via induction of IL-10 production and down-regulation of B7-2 costimulator molecules [22]. PACAP also induces histamine release in skin mast cells, in common with VIP.

### 2.5.7 POMC-derived peptides

POMC is a neurohormone, produced not only in the pituitary gland but also by various cell types, which is synthesized as a large prohormone and subsequently cleaved by specific serine proteases called prohormone convertase (PC)1 and PC2. The active peptide hormones formed include melanocyte-stimulating hormones (α-, β-, and γ-MSH), adrenocorticotrophic hormone (ACTH), corticotropin-like intermediate lobe peptide (CLIP), α-lipotrophic hormone (α-LPH) and β-endorphin (β-EP). In the skin, POMC-peptides are constitutively present in KCs, melanocytes and LCs in the epidermis, and in fibroblasts and dermal microvascular endothelial cells in the dermis. Levels of the hormones are up-regulated by various stimuli such as IL-1, TNF-α, UVB irradiation and phorbol esters. Conversely, TGF-β has been shown to down-regulate mRNA specific for POMC in fibroblasts. In addition, POMC-peptides have been detected in various immune cells such as lymphocytes, macrophages and splenocytes.

POMC-peptides exert pleiotropic effects, which are mediated by five G-protein-coupled melanocortin (MC) receptors whose expression varies in different tissues and which have different affinities to individual POMC-peptides. The human MC-1R binds α-MSH with the highest affinity and is widely expressed by inflammatory (T and B cells), antigen-presenting (macrophages, dendritic cells), epidermal (KCs, melanocytes) and dermal (fibroblasts, endothelial) cells. MC-1R is also expressed by cutaneous adnexal structures including hair follicles, sweat glands and sebaceous glands. Expression of MC-1R can be up-regulated by IL-1, UV irradiation and α-MSH itself. MC-2R binds specifically to ACTH, whilst MC-3R and MC-4R are mainly expressed within the central nervous system. MC-5R has been detected at a low level in cutaneous sebaceous glands and can be up-regulated by stimulation of sebocytes with α-MSH.

### 2.5.7.1 Modulation of immune cell function

POMC-peptides, particularly  $\alpha$ -MSH, are potent modulators of inflammatory and immune cell responses [23].  $\alpha$ -MSH inhibits proinflammatory (IL-1, TNF- $\alpha$ , IL-6) and immunomodulatory (IFN- $\gamma$ , IL-4, IL-13) cytokine production, but up-regulates the production of the anti-inflammatory cytokine, IL-10. The hormone also down-regulates the expression of MHC Class I, costimulatory CD86 and CD40 molecules and ICAM-1 on monocytes and dendritic cells, and inhibits macrophage production of the cytotoxic compound, NO. In addition,  $\alpha$ -MSH inhibits the activation of the nuclear transcription factor NF- $\kappa$ B by various inflammatory agents including IL-1, TNF- $\alpha$  and LPS. These immunomodulatory and anti-inflammatory effects appear to be mediated by the C-terminal tripeptide of  $\alpha$ -MSH.

In mouse models of contact hypersensitivity *in vivo*, systemic or topical application of  $\alpha$ -MSH or its C-terminal tripeptide, prior to sensitisation with contact allergens, inhibits both the sensitisation and elicitation phases of contact hypersensitivity and induces hapten-specific tolerance via effects on dendritic cell function. These effects are mediated by the generation of IL-10-producing suppressor T cells expressing cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), a B7-binding molecule which, when cross-linked, causes decreased T cell proliferation due to down-regulated IL-2 production.

$\alpha$ -MSH (and ACTH) also modulates IL-4 and anti-CD40-mediated release of IgE by B cells *in vitro*. This may result in an increase in antibody production when the neuropeptides are present at physiological concentrations, or, conversely, inhibition at higher peptide doses. In a mouse model of allergic airway inflammation, systemic treatment with  $\alpha$ -MSH *in vivo* resulted in a marked reduction in allergen-specific IgE production, eosinophil influx and IL-5 production, which was mediated by IL-10 production.

$\alpha$ -MSH also inhibits histamine release and decreases mRNA specific for IL-1, TNF- $\alpha$  and lymphotactin (XCL1, a chemokine for T cells), in mast cells.

### 2.5.7.2 Modulation of cutaneous cell function

In the skin, the production of chemokines such as CXCL8 (IL-8) and CXCL1 (GRO- $\alpha$ ) by KCs in response to stimulation by IL-1 is suppressed by  $\alpha$ -MSH. Partial blockage of IL-1-induced chemokine production by sebocytes has also been reported. In contrast,  $\alpha$ -MSH synergises with IL-1 to stimulate dermal microvascular endothelial cells to secrete increased levels of CXCL8, but down-regulates the LPS-induced expression of adhesion molecules essential for leukocyte recruitment and extravasation across the vessel wall, such as ICAM-1, VCAM-1 and E-selectin.

$\alpha$ -MSH also plays an important role in pigmentation of hair, stimulating follicular melanocytes to proliferate and produce eumelanin (brown pigment) via the MC-1R receptor.

## 2.6 Proteinase-activated receptors

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Proteinase-activated receptors (PARs) constitute a novel subset of seven-transmembrane, G-protein-coupled receptors with a unique mechanism of activation [reviewed in ref 24]. Instead of stimulation via binding of a ligand, activation of PAR is initiated by proteolytic cleavage of the N-

terminus of the receptor by serine proteases exposing a tethered ligand that binds and autoactivates the receptor. Four PARs have been described, three of which (PAR-1, -3, -4) are sensitive to thrombin, a trypsin-like serine protease that mediates the formation of fibrin from fibrinogen in the coagulation cascade. In contrast, PAR-2 is sensitive to trypsin and tryptase, and other serine proteases that are tissue-specific. Both PAR-1 and PAR-2 have been described in skin and are involved in regulation of cutaneous inflammation.

### 2.6.1 PAR-1

PAR-1 is expressed by KCs in the epidermis, and fibroblasts, vascular smooth muscle and endothelial cells in the dermis. Thrombin stimulates  $\text{Ca}^{2+}$  influx, proliferation and IL-6 production, but inhibits differentiation of KCs via its effects on PAR-1. Similarly, PAR-1 activation of fibroblasts induces increased proliferation, IL-6 and GM-CSF production.

Activation of endothelial cells is an important part of the coagulation and wound healing processes. Thrombin, released from platelets, induces  $\text{Ca}^{2+}$  influx and stimulates the release of von Willebrand factor (an adhesive protein), the cell surface redistribution of P-selectin, and increased expression of ICAM-1, VCAM-1 and E-selectin. Thrombin also stimulates mitogenesis, cell contraction, and increased permeability of endothelial cells, and increases NO, PDGF and ET-1 production. The VEGF receptors, *kdr* and *flt-1*, on endothelial cells are also up-regulated.

### 2.6.2 PAR-2

KCs, endothelial cells, hair follicles, myoepithelial cells of sweat glands, DDC, and sensory afferent nerves in the skin all express PAR-2. The main endogenous activator of PAR-2 in skin is most likely tryptase, a chymotrypsin-like protease, which is abundant in mast cells. Although trypsinogen, the precursor of trypsin, is present in skin (produced by endothelial cells during inflammation), it has not been established whether specific enteropeptidases required for processing of trypsin are also available.

PAR-2 is expressed at higher levels in the more differentiated granular layer than in the basal and suprabasal KCs of the epidermis, but is up-regulated throughout the epidermis by UV irradiation. PAR-2 activation of KCs results in transient cytosolic  $\text{Ca}^{2+}$  mobilisation but, in contrast to PAR-1 stimulation, proliferation is inhibited even in the presence of growth factors. Differentiation of KCs, and calcium- or IFN- $\gamma$ -induced transglutaminase type 1 and involucrin synthesis are also inhibited, whilst production of IL-6, IL-8 and GM-CSF are increased. In addition, activation of PAR-2 stimulates phagocytosis and melanosome uptake by KCs.

PAR-2 activation of endothelial cells by agonists such as tryptase induces  $\text{Ca}^{2+}$  mobilisation, increases IL-6 and IL-8 production, and activates NF- $\kappa$ B transcription factor.

In a mouse model of experimentally induced allergic and toxic contact dermatitis, functions such as ear swelling, plasma extravasation and leukocyte adherence were shown by gene deletion to be dependent upon PAR-2 expression, supporting a proinflammatory role for the receptor *in vivo* [25]. These effects were mediated by NO and did not involve NF- $\kappa$ B activation. Furthermore, intralesional injection of endogenous PAR-2 agonists induced enhanced and prolonged itch in patients with atopic dermatitis via stimulation of neuronal PAR-2 [26].

## 2.7 Vanilloid receptor-1

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Vanilloid receptor-1 (VR-1) is a non-selective cation channel, which acts a polymodal receptor responding not only to vanilloids such as capsaicin (a pain inducer), but also to heat and protons (reduced pH), conditions present during tissue injury. In the skin, VR-1 is expressed on sensory nerve fibres and on various cell types such as KCs (predominately basal), mast cells, dermal blood vessels, hair follicles, differentiated sebocytes, sweat gland ducts and the secretory part of eccrine sweat glands [27].

Activation of VR-1 on KCs by capsaicin induces a dose-dependent influx of  $\text{Ca}^{2+}$ , and an increase in CXCL8 (IL-8),  $\text{PGE}_2$  and COX-2 (see Section 3.2.2.1) production which can be attenuated by the VR-1 antagonist, capsazepine [28]. Thus activation of epidermal VR-1 may play a role in the induction of inflammation in response to noxious chemical stimuli.

## Summary

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The humoral components of the skin immune system consist of complement components, polyunsaturated fatty acid metabolites, secretory immunoglobulins, cytokines and chemokines, and neuropeptides.

Complement components produced by KCs include C3 and B, which are involved in the alternative complement activation pathway. Simultaneous production of complement regulatory proteins ensures that the KCs are protected from complement-mediated damage. Linoleic acid and arachidonic acid are produced by KCs and metabolised in the epidermis to 13-HODE, and PGE<sub>2</sub> or 15-HETE, respectively. These metabolites are involved in regulation of epidermal cell function. Small amounts of LTB<sub>4</sub>, which has various functions including acting as a chemoattractant for T cells and PMNs, are also produced from arachidonic acid. Secretory components (which bind IgA) and/or bound IgA, are present on KCs, and sweat and sebaceous glands forming part of the skin's defence system.

Cytokines produced by KCs have paracrine effects on LCs, T cells, endothelial cells and fibroblasts, which may, in turn, exert reciprocal effects on KC function. Functional effects induced by KC-derived cytokines on target cells include modulation of viability, proliferation, cytokine production, expression of adhesion molecules and trafficking.

KCs produce a range of CXC and CC chemokines, which attract T cells, LCs and other immune cells to the skin.

Neuropeptides are released from sensory or autonomic nerve fibres in the skin, and by epidermal and dermal cells, and include substance P, CGRP, neuropeptide Y, somatostatin, VIP, PACAP, POMC-derived peptides, PAR and VR-1. Several cell types in the skin express specific receptors for these molecules, which can modulate various immune and inflammatory functions.

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