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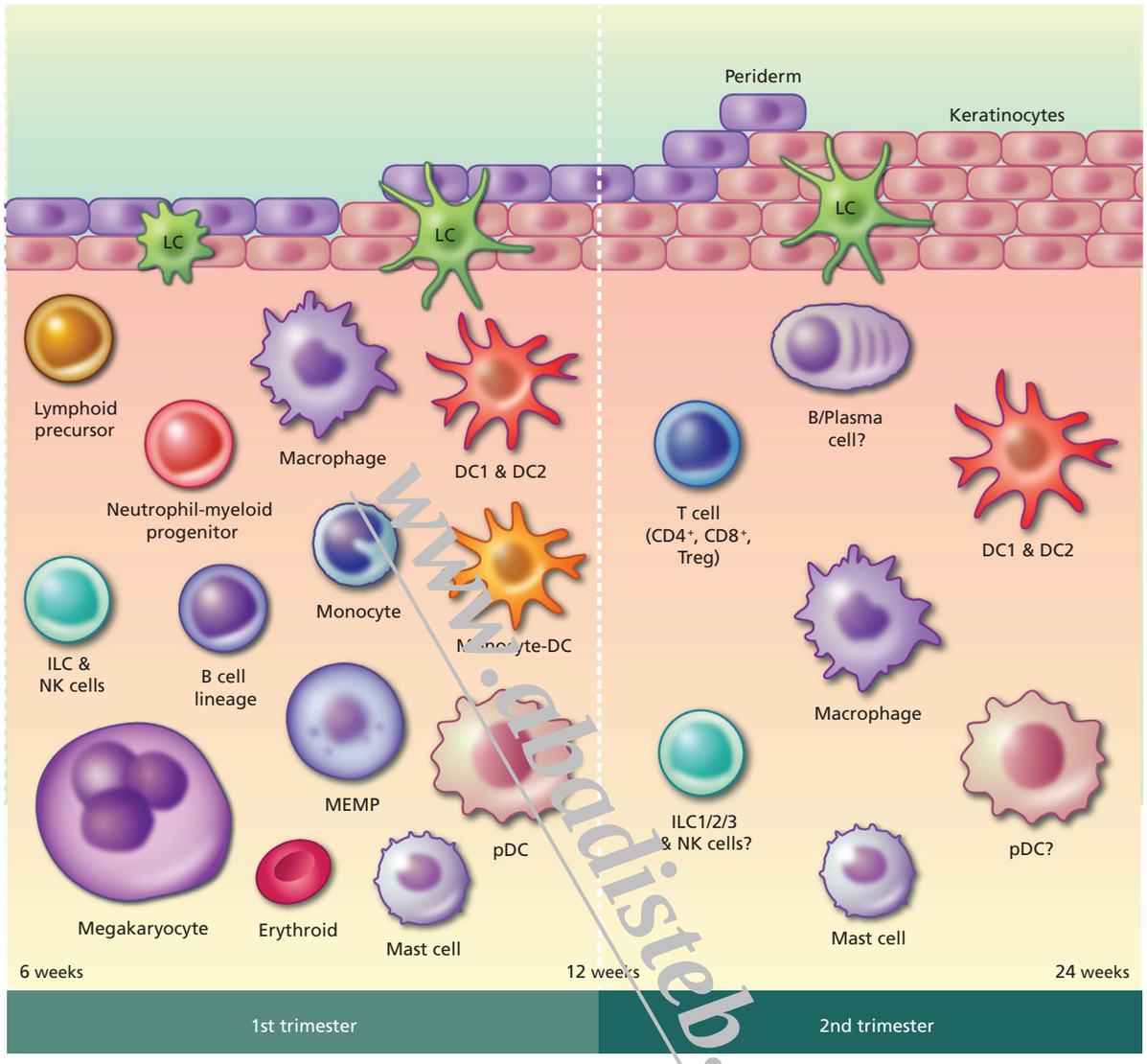


Figure 2.4 Overview of the immune cells present in first- and second-trimester human skin. ?, unknown; DC, dendritic cell; ILC, innate lymphoid cell; LC, Langerhans cell; MEMP, megakaryocyte-erythroid-mast cell progenitor; Monocyte-DC, monocyte-DC hybrid; NK, natural killer. Adapted from Botting and Haniffa 2020 **[3]** with permission from Wiley.

rudiments occurs at about 9 weeks in the regions of the eyebrow, upper lip and chin (Figure 2.5). Mesenchymal cells, derived from the dermomyotome, populate the skin and interact with the overlying epidermis to induce the formation of hair placodes [6]. Key components of the mesenchymal signals to produce hair follicles include FGFs and BMP-inhibitory factors such as Noggin; excessive BMP stimulation can reduce hair follicle density. The epidermal response to form the hair placode is generated by Wnt signals such as Wnt10b and sonic hedgehog (Shh), which also has a key role in the formation of the dermal papilla [7]. After it is formed, the dermal papilla sends further signals to transform the placode into a hair follicle. At the centre of the signalling cross-talk is the bipartite transcription factor composed of lymphoid enhancer-binding factor 1 (LEF-1) and stabilised β-catenin, which is essential for hair follicle formation. Hair follicle development is also influenced by Smads, a group of signalling mediators and antagonists of the transforming growth factor β (TGF-β) superfamily. Smad-4 affects hair follicle differentiation by mediating BMP signalling; Smad-7 affects hair follicle development and differentiation by blocking TGF-β/activin/BMP pathways [8]. Skin development is governed by complex, balanced waves of gene activation and silencing; cross-talk between small non-coding micro-RNAs and messenger RNAs is very important for the coordination of signal transduction and transcriptional activation [9].

Signalling responses differ between follicular and interfollicular epidermis: BMP signalling is active in the interfollicular epidermis and is both an epidermis-promoting signal as well as a follicle-inhibiting signal; epidermal growth factor receptor (EGFR) signalling may have a similar role in governing follicle density. As hair follicles mature to form inner and outer root sheaths, several signalling pathways are involved, including Wnt, Notch and BMP receptors. There are also marked changes in certain cell adhesion proteins, notably E-cadherin and P-cadherin. The hair follicles

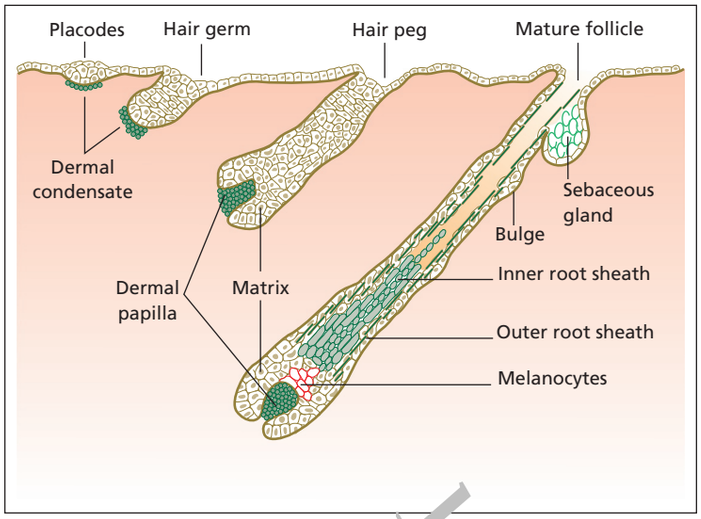


Figure 2.5 Embryonic stages of hair follicle morphogenesis.

are arranged in patterns, usually in groups of three. It appears that the first follicles develop over the surface at fixed intervals of between 274 and 350 μm. As the skin grows, these first hair germs become separated, and new rudiments develop between them when a critical distance, dependent on the region of the body, has been reached. There is no large-scale destruction of follicles during postnatal development, only a decrease in actual density as the body surface increases; nor do any new follicles develop in adult skin. In interfollicular epidermis, the undersurface of the epidermis is smooth, but during the fourth month, at the same time as the hair follicle starts to develop, it becomes irregular.

Sebaceous glands first appear as hemispherical protuberances on the posterior surfaces of the hair pegs. The cells contain moderate amounts of glycogen, but soon the cells in the centre lose this, and become larger and accumulate droplets of lipid. The sebaceous glands become differentiated at 13–15 weeks and are then large and functional. The sebum forms part of the vernix caseosa. At the end of fetal life, sebaceous glands are well developed and generally large. After birth, the size is rapidly reduced, and they enlarge to become functional again only after puberty. The molecular signals that induce sebaceous gland differentiation involve the c-Myc transcription factor as well as the adipogenic transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ) [10].

Eccrine glands start to develop on the palms and soles at about 3 months, but not over the rest of the body until the fifth month [11]. In embryos of 12 weeks, the rudiments of eccrine sweat glands are first identifiable as regularly spaced undulations of the developing epidermis. Cells that go on to form the eccrine sweat glands are oblong, palisading and lie closely together, but otherwise they do not differ from the rest of the developing basal epidermis. By 14–15 weeks, the tips of the eccrine sweat gland rudiments have penetrated deeply into the dermis and have begun to form the coils. In the overlying epidermis, columns of cells that are destined to form the intraepidermal sweat ducts are recognisable. Each column is composed of two distinct cylindrical layers, comprising two inner cells that are elongated and curved so that they embrace the inner cylinder. The intraepidermal duct appears to form by the coalescence of groups of intracytoplasmic cavities formed within two adjacent inner cells. In the intradermal segment, the lumen forms by dissolution of the desmosomal attachment plaques between the cells that compose the inner core of the eccrine duct germ.

Nails begin to develop in the third month. Key signalling events in nail development involve the R-spondin family of transcription factors [12]. In fetuses at 16–18 weeks (crown to rump length 120–150 mm), keratinising cells from both dorsal and ventral matrices can be distinguished. Melanocytes take their origin from the neural crest. This can be identified in early human embryos, but the elements arising from it soon lose themselves in the mesenchyme, and pigmented melanocytes cannot be identified, even in darker skin fetuses, before 4–6 months of gestation. However, dopa-positive melanocytes can be demonstrated earlier. Langerhans cells are derived from the monocyte–macrophage–histiocyte lineage and enter the epidermis at about 12 weeks. Merkel cells appear in the glabrous skin of the fingertips, lips, gingiva and nail bed, and in several other regions, around 16 weeks.

Although some cells of the dermis may migrate from the dermatome (ventrolateral part of the somite) and take part in the formation of the skin, most of the dermis is formed by mesenchymal cells that migrate from other mesodermal areas [13]. These mesenchymal cells give rise to the whole range of blood and connective tissue cells, including the fibroblasts and mast cells of the dermis and the fat cells of the subcutis. In the second month, the dermis and subcutis are not distinguishable from each other but distinct collagen fibres are evident in the dermis by the end of the third month. Later, the papillary and reticular layers become distinct and, at the fifth month, the connective tissue sheaths are formed around the hair follicles. Elastic fibres are first detectable at 22 weeks.

Epidermal and adnexal structures

The normal epidermis is a terminally differentiated, stratified, squamous epithelium. The major cell type, making up 95% of the total, is the *keratinocyte*, which moves progressively from attachment to the epidermal basement membrane towards the skin surface, forming several well-defined layers during its transit [1]. Thus, on simple morphological grounds, the epidermis can be divided into four distinct layers: *stratum basale* or *stratum germinativum*, *stratum spinosum*, *stratum granulosum* and *stratum corneum*. The term *Malpighian layer* includes both the basal and spinous cells. Other constitutive cells within the epidermis include melanocytes, Langerhans cells and Merkel cells (Figure 2.6).

The stratum basale is a continuous layer that is generally only one cell thick. The basal cells are small and cuboidal (10–14 μm in diameter) and have large, dark-staining nuclei and dense cytoplasm that contains many ribosomes and dense tonofilament bundles. Immediately above the basal cell layer, the epibasal keratinocytes enlarge to form the spinous/prickle cell layer or stratum spinosum (Figure 2.7).

The stratum spinosum is succeeded by the stratum granulosum or granular layer, which contains intracellular granules of keratohyalin. At high magnification, the dense mass of keratohyalin

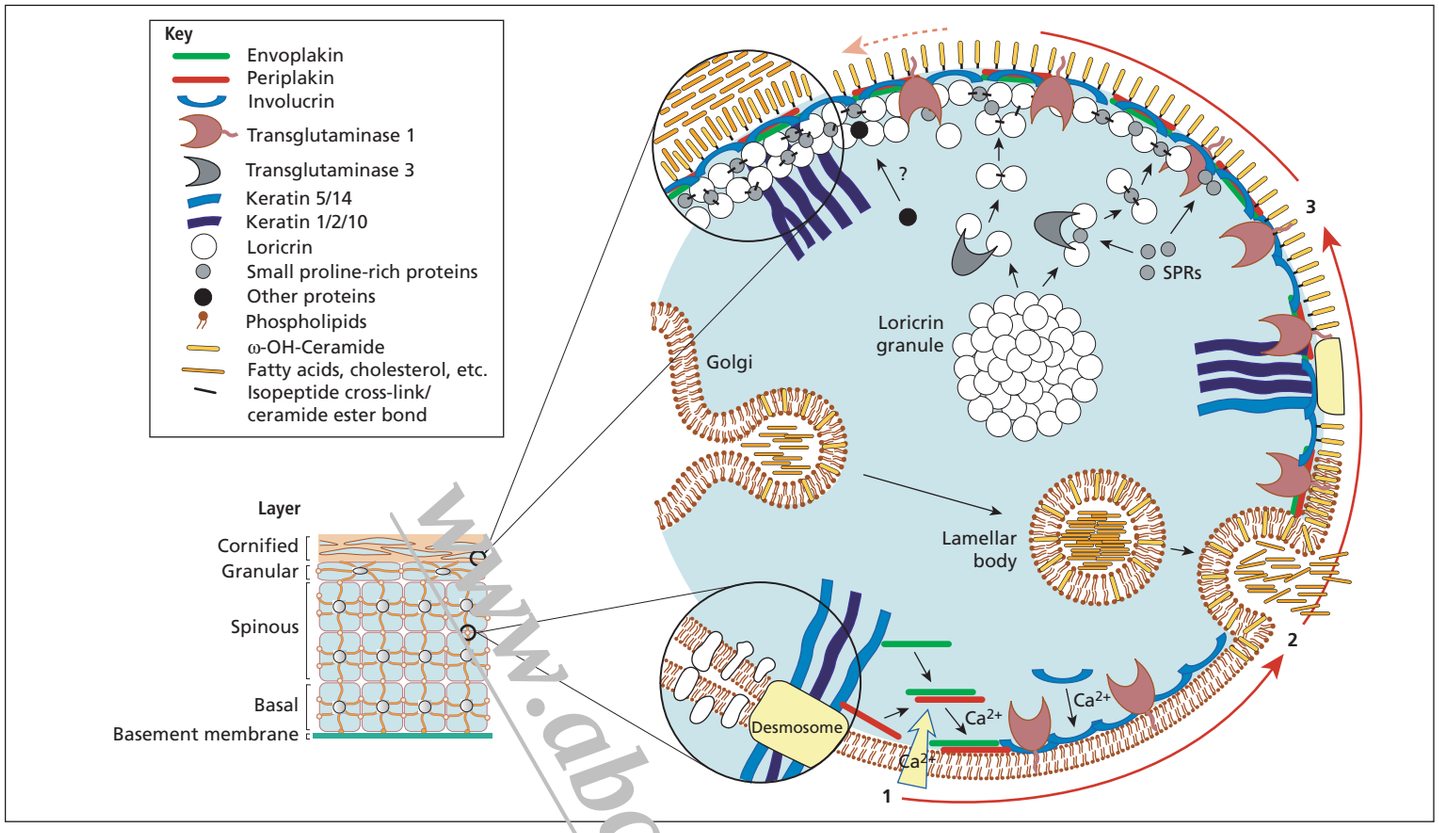


Figure 2.9 Assembly of the epidermal cornified cell envelope. In response to increasing intracellular calcium, an internal scaffold of desmosomal proteins is made along the plasma membrane. The contents of lamellar bodies (ceramides and other fatty acids, cholesterol and cholesterol esters) are released into the extracellular milieu to form a lipid membrane. The developing envelope is then added to and reinforced by the recruitment of various proteins, including loricrin, small proline-rich proteins (SPRs), other desmosomal remnants and attached keratin filaments. The resulting cornified cell envelope is durable and flexible and provides important mechanical and barrier functions.

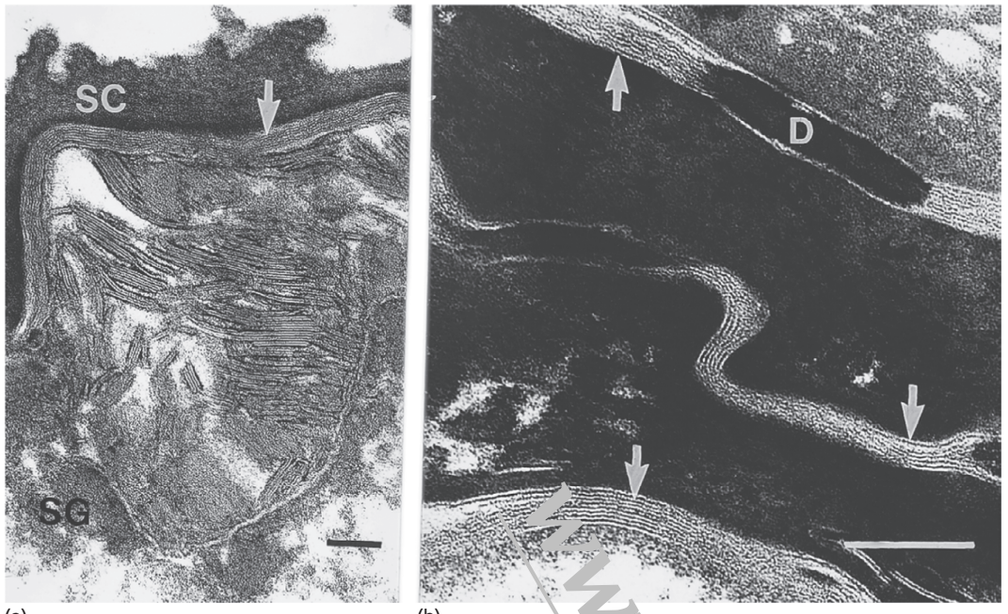
precursor *involucrin*, following the action of a specific epidermal transglutaminase also synthesised in the high stratum spinosum (Figure 2.10). Many of the proteins involved in terminal differentiation are derived from a cluster of about 25 genes located within a *c*.2 Mb region on the long arm of chromosome 1. Termed the epidermal differentiation complex (EDC), these coding elements are derived from at least three families of structurally, functionally and evolutionarily related genes. Together, the EDC proteins have roles in structural integrity, signal transduction and cell cycle progression and may be primarily or secondarily disrupted in several inflammatory or neoplastic disorders.

The process of desquamation involves degradation of the lamellated lipid in the intercellular spaces and loss of the residual intercellular desmosomal interconnections [5]. In palmoplantar skin there is an additional zone, also electron-lucent, the *stratum lucidum*, between the granulosum and corneum. These cells are still nucleated and may be referred to as 'transitional' cells.

Keratinocytes

The filamentous cytoskeleton of all mammalian cells, including epidermal keratinocytes, is composed of actin-containing microfilaments approximately 7 nm in diameter; tubulin-containing microtubules 20–25 nm in diameter; and filaments of intermediate size, 7–10 nm in diameter, known as intermediate filaments. There are six types of intermediate filaments: keratins in epithelial cells; vimentin within mesenchymal cells; glial filament acidic protein (GFAP) in glial cells; neurofilaments in neurons; desmin in muscle cells and peripherin in peripheral nerves. The nuclear matrix proteins, nuclear lamins A, B and C, are also intermediate filaments. The polypeptide building blocks of all intermediate filaments have a similar backbone structure of a classic α-helical region with heptad repeats, having four separate helical zones with interhelical linker sequences, and non-helical carboxy- and amino-terminals. There are 70 intermediate filament genes (including those encoding keratins, desmins and lamins), which are now known to be associated with numerous human diseases, including skin blistering, muscular dystrophy, cardiomyopathy, premature ageing syndromes, neurodegenerative disorders and cataract [**1**,2].

The human genome possesses 54 functional keratin genes located in two compact gene clusters, as well as many non-functional pseudogenes scattered around the genome [3]. Keratin genes are very specific in their expression patterns. Each one of the many highly specialised epithelial tissues has its own profile of keratin gene expression. Hair and nails express modified keratins, containing large amounts of cysteine which forms numerous chemical cross-links to further strengthen the cytoskeleton. The



(a) (b)

Figure 2.10 Electron micrograph showing the location of epidermal lipids by ruthenium oxide staining. (a) Extrusion of lamellar body lipids or sheets can be seen at the interface between the stratum granulosum (SG) and stratum corneum (SC). Scale bar 0.1 μm. (b) Sheets of lipid bilayers (arrowed) are present in the intercellular spaces of the SC. Some regions show a repetitive pattern of staining. D, desmosome. Scale bar 0.1 μm. Courtesy of Dr M. Fartasch, Department of Dermatology, University of Erlangen, Germany.

genes encoding individual keratins fall into two families: type I (acidic) and type II (basic). Mapping the tissue distribution of keratins shows coexpression of partner acidic–basic pairs in a cell- and tissue-specific manner. Heterodimers are assembled into higher order protofibrils and protofilaments by an antiparallel stagger of some complexity.

Simple epithelia are characterised by the keratin pair K8/K18, and the stratified squamous epithelia by K5/K14 (Figure 2.11). In addition, stratified squamous epithelia express up to four other keratin pairs during epithelial differentiation. In skin, suprabasal keratins K1/K10 are characteristic of epidermal differentiation. In the stratum granulosum, release of filaggrin from the keratohyalin granules forms macrofibres. Retinoid levels, growth factors and hormones may regulate keratin gene expression. Mesenchymal signals may also direct or permit intrinsic patterns of keratinocyte differentiation. K15 is expressed in basal keratinocytes of the hair follicle bulge region at the site of pluripotential stem cells. K9 and K2 expression is site restricted in skin: K9 to the palmoplantar epidermis and K2 to the superficial interfollicular epidermis. Apart from their structural properties, keratins may also have direct roles in cell signalling, the stress response and apoptosis [**1**,4]. In epidermal hyperproliferation, as in wound healing and psoriasis, the expression of suprabasal keratins K6/K16/K17 is rapidly induced.

Currently, at least 21 of the 54 known keratins (28 type I and 26 type II) have been linked to monogenic genetic disorders, and some have been implicated in more complex traits such as idiopathic liver disease or inflammatory bowel disease [5]. The first genetic disorder of keratin to be described was epidermolysis bullosa simplex, which involves mutations in the genes encoding K5 or K14. About half of the 54 keratin genes are expressed in the hair follicle (trichocyte 'hard' keratins), although only a minority of these have been linked to human genetic disorders (monilethrix, hair–nail ectodermal dysplasias, pseudofolliculitis barbae and woolly hair) [6].

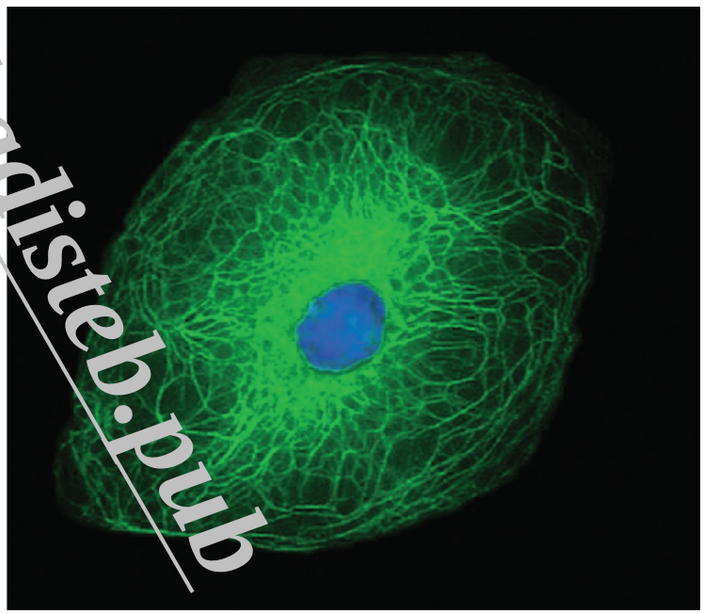


Figure 2.11 Structural organisation of the keratin filament network within a keratinocyte. Courtesy of Professor W. H. I. McLean, University of Dundee, UK.

Eccrine and apocrine glands

Human sweat glands are generally divided into two types: apocrine and eccrine [1]. The eccrine gland is the primary gland responsible for thermoregulatory sweating in humans. Eccrine sweat glands are distributed over nearly the entire body surface. Sweat glands become identifiable in the palms and soles in the 16th fetal week, and in the rest of the body from the 22nd week onwards. The number of sweat glands in humans varies greatly, ranging from 1.6 million

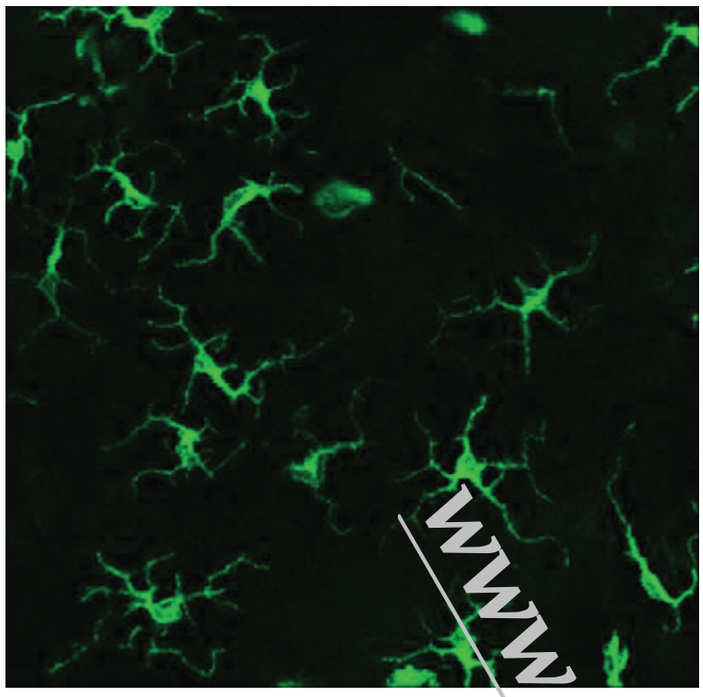


Figure 2.17 Dendritic appearance of epidermal Langerhans cells. Exposure to antigen provokes an increased movement of Langerhans cells as well as direct cell–cell contact between Langerhans cells. Courtesy of Dr R. Mohr, University of Toledo, Ohio, USA.

gland and in the epithelium of the crypts of the human tonsil. The discovery of similar granules in cells in the dermis in histiocytosis X resulted in the renaming of this condition as Langerhans cell histiocytosis.

Immune surveillance

Besides the antigen detection and processing role of epidermal Langerhans cells, cutaneous immune surveillance is also carried out in the dermis by an array of tissue-resident T cells, macrophages and dendritic cells (Figure 2.20) [1]. These immune sentinel and effector cells can provide rapid and efficient immunological backup to restore tissue homeostasis should the epidermis be breached. Resident memory T cells act as alarm-sensor cells or cytotoxic cells, often persisting in the skin for a long time, and can be reactivated upon reinfection with the same antigen. The dermis contains a very large number of resident T cells; remarkably, there are approximately 2×10^{10} skin-resident T cells, which is twice the total number of T cells in the circulating blood [2,3]. There are several distinct populations of dermal dendritic cells; some have potent antigen-presenting capacities, others have low antigen-presenting capacity but the potential to develop into CD1a+ and langerin-positive Langerhans cells, while some are pro-inflammatory.

The cellular diversity of dermal immune sentinels is reflected in some flexibility or plasticity in function. For example, immature dendritic cells, including dermal dendritic cells, can be phagocytic, which is a cellular function usually attributed to macrophages [4]. Alternatively, macrophages, which normally are phagocytic cells, can also be potent antigen-presenting cells for CD8+ T cells. This means that tissue-resident mononuclear sentinels of the dermis are likely to exist in a pluripotent state. Depending on microenvironmental factors and cues, they may acquire an antigen-presenting mode, a migratory mode or a tissue-resident phagocytic mode.

Mast cells

Mast cells were first described by Ehrlich in 1877, who distinguished them from other connective tissue cells by their ability to stain metachromatically with basic aniline dyes. Mast cells are larger than eosinophils and basophils. They occur in most tissues, but are particularly numerous in the skin, bronchus, nasal mucosa and gut. In the skin, mast cells are distributed close to blood vessels, nerves and appendages, and are most numerous in the subpapillary dermis, in the region of the superficial dermal vascular plexus. There are about 7000 mast cells/mm^3 in normal skin.

Dermal mast cells are ovoid or spindle shaped, mononuclear or occasionally binuclear, and only rarely show signs of mitosis in normal skin. Their major distinguishing feature is the presence of numerous, round, cytoplasmic granules (Figures 2.21 and 2.22). Mast cells are heterogeneous and fall into two main types – connective tissue and mucosal – which can be differentiated by their morphology, tissue distribution, histochemical characteristics and responses to degranulating agents. Solubility of the granules in formaldehyde and the content of neutral proteinase, namely tryptase and chymase (chymotryptic proteinase), will vary according to the type of cell. For example, human foreskin mast cells contain both proteinases, whereas mast cells in intestinal mucosa and the lung contain mainly tryptase [1].

Human mast cells arise from CD34+ pluripotent stem cells in the bone marrow. They then circulate in the blood as precursors and home to tissues where they mature under the influence of stem cell factor (SCF) and local cytokines and other factors. Mast cell growth and differentiation are also influenced by several other cytokines, including interleukin 3 (IL-3), -4, -6, -9, -10 and nerve growth factor. Mast cells are long lived and may proliferate in association with immunoglobulin E (IgE)-dependent activation and in the presence of IL-4 [2].

Kit (CD117), expressed on haematopoietic stem cells and progenitor cells, is the tyrosine kinase transmembrane receptor for SCF that is involved in the differentiation of both myeloid and lymphoid lineages. While Kit is downregulated on other bone marrow-derived cells during their differentiation, Kit remains highly expressed on mast cells and is critical for many mast cell functions such as survival, differentiation, chemotaxis and enhancement of signalling events during mast cell activation. The importance of Kit is shown by the finding of activating mutations in the *KIT* gene in patients with urticaria pigmentosa [3].

Upon activation of mast cells via cross-linking of the high-affinity IgE receptor (FcεRI) or non-IgE-mediated activation through complement receptors or Toll-like receptor (TLR) activation, mast cells can release histamine, serotonin and proteases as well as newly synthesised leukotrienes, prostaglandins, cytokines and chemokines. In addition to IgE-mediated activation, human mast cells exposed to interferon γ (IFN-γ) can be activated following IgG-mediated aggregation of FcγRI to release similar mediators.

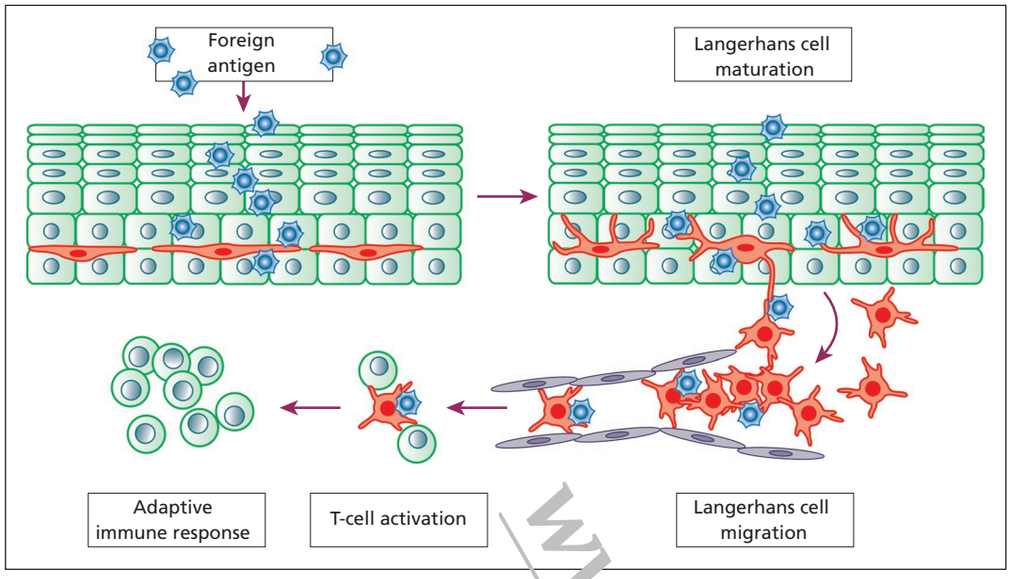


Figure 2.18 When exposed to foreign antigen, the activity of resting Langerhans cells increases, and the cells mature. Antigen is then processed and transported to the lymph nodes. T cells are then activated, and an immune response is triggered.

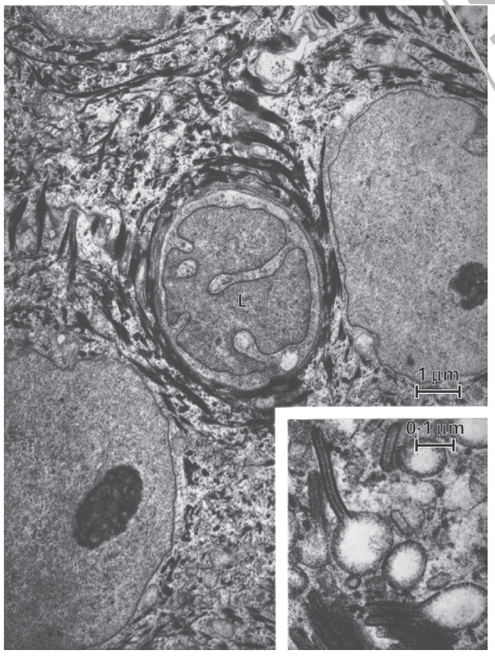


Figure 2.19 Langerhans cell (L) with its characteristically indented nucleus, situated between keratinocytes. The inset shows Langerhans cell granules with racquet-shaped profiles. Courtesy of Professor A. S. Breathnach.

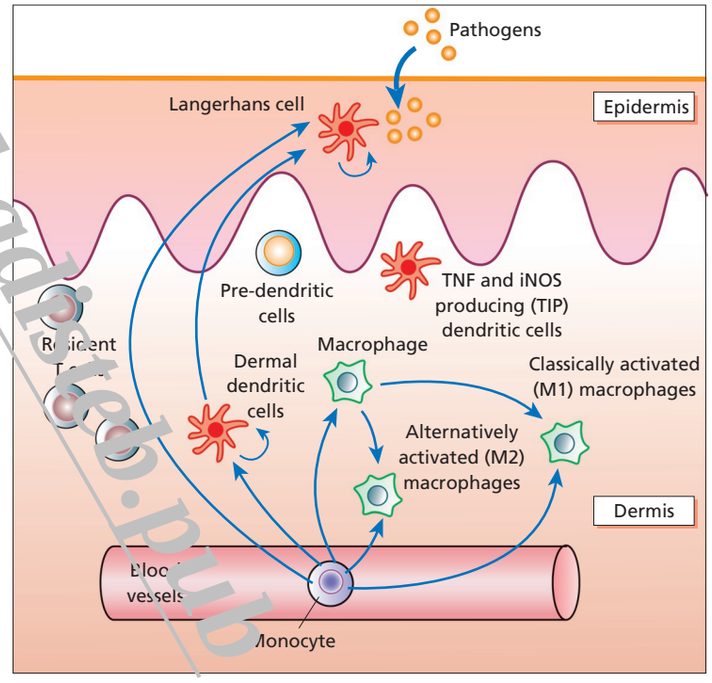


Figure 2.20 Immune surveillance in normal skin is carried out by an array of skin-based dendritic cells, macrophages and resident T cells. iNOS, inducible nitric oxide synthase; TNF, tumour necrosis factor.

Additional IgE-independent mast cell triggers have been described, including SCF, complement (C3a and C5a), neuropeptides (substance P), adenosine, TLR and scavenger receptors.

Mast cell products may both induce an immediate reaction and contribute to a late phase reaction. The immediate phase reaction occurs within minutes of FcεRI cross-linking and its consequences are referred to as an immediate hypersensitivity reaction. Late phase reactions peak 6–12 h following antigen challenge and are associated with cytokines and chemokines from eosinophils, neutrophils and basophils that have been secondarily recruited. Mast cell activation results in increased vascular permeability and smooth muscle contraction, as well as fibroblast deposition of collagen, induction of B cells to class switch to synthesise IgE, basophil histamine release, recruitment of neutrophils and eosinophils, and promotion of T cells to a Th2 phenotype.

Mast cells play an important role in both adaptive and innate immunity and contribute to the skin pathology seen in contact dermatitis, atopic eczema (AE), immunobullous disease, scleroderma and chronic graft-versus-host disease; they also have a

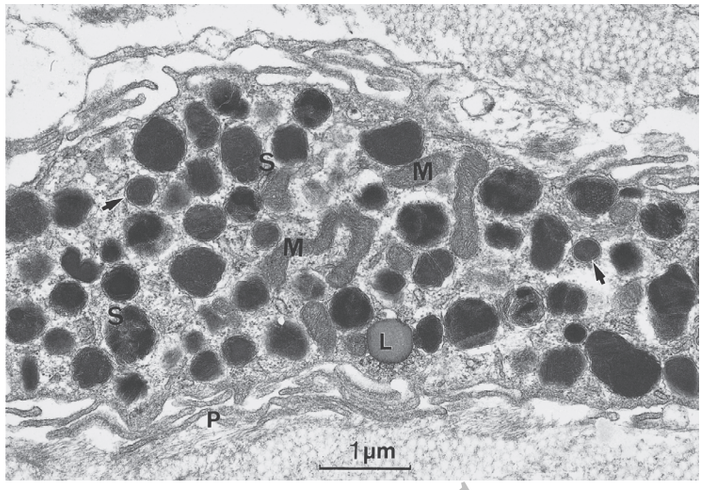


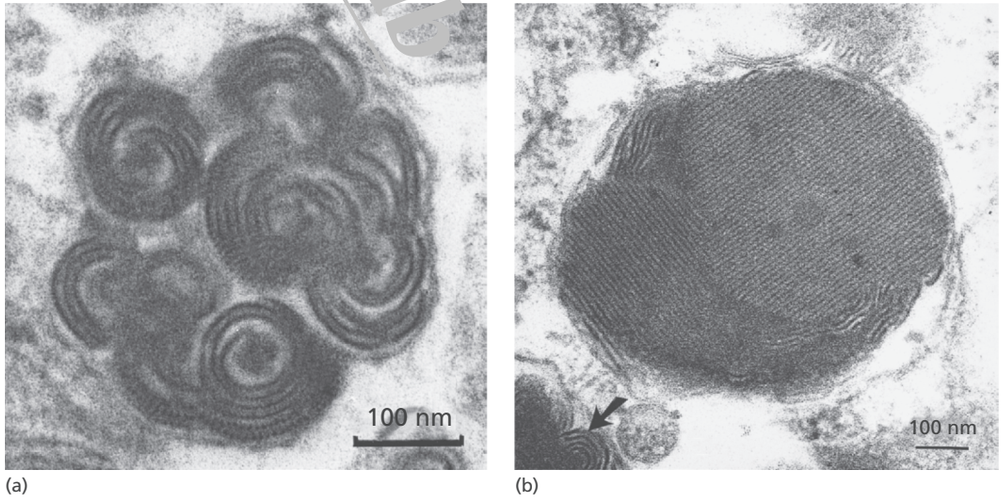
Figure 2.21 Part of a human skin mast cell showing characteristic granules, some with scroll-like profiles (S). Arrows indicate perigranular membrane; L, lipid droplet; M, mitochondria; P, peripheral processes. Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.

role in immune regulation [4]. In AE, there is an increase in mast cell numbers in lesional skin. Mast cells reside in the papillary dermis and undergo migration through the basal lamina into the epidermis. Although overall levels of histamine are not increased in AE, tryptase and activation of proteinase-activated receptor-2 (PAR-2) may contribute to the pruritus seen in AE, as tryptase is reported to be increased up to fourfold in AE patients and PAR-2 expression is markedly enhanced on primary afferent nerve fibres in skin biopsies from patients with AE. Chymase may play a role in eliciting and maintaining chronic inflammation in AE by increasing spongiosis and compromising the skin barrier. Mast cell–nerve interactions may also play a role in promoting inflammation in AE. There is an increased number of contacts between mast cells and nerves in both lesional and non-lesional skin, which may lead to inflammation mediated by neuropeptides such as substance P, calcitonin gene-related peptide, vasoactive intestinal peptide and nerve growth factor.

Melanocytes

Melanocytes are pigment-producing cells located in the skin, inner ear, choroid and iris of the eye. In skin, melanocytes are dendritic in shape, are mainly located at the dermal–epidermal junction, and connect to the basement membrane by a dense plate structure that shares similarities with hemidesmosomes. Melanocytes in adult skin and hair develop from embryonically derived melanocyte precursors called melanoblasts [1]. During development, melanoblasts emerge from a subset of neural crest cells and migrate to the interfollicular skin and to developing hair follicles (Figure 2.23). In the hair follicle, melanocytes are divided into two distinct populations: differentiated melanocytes, located in the hair bulb where they provide melanin to the growing hair matrix, and melanocyte stem cells, located at the hair bulge. Melanocyte stem cells are typically quiescent but undergo cyclical proliferation, differentiation and migration. Melanocytes maintain their polarity similarly to neuronal cells, driving cellular asymmetry to control, for example, protrusion and spine formation, organelle transport and metastasis. Polarity regulators may be relevant to melanocyte architecture, function and quiescence and dysregulation therein may be implicated in both hypopigmentation and malignant melanoma. The life cycles of the follicular melanocytes and melanocyte stem cells are closely related to the cyclical nature of the hair follicle, and during anagen new melanocytes are generated from the pool of slow-proliferating melanocyte stem cells [2].

Melanin is synthesised and accumulates within the melanosome, an organelle within the melanocyte. In the skin and hair, two forms of melanin pigment are produced: brown/black eumelanin and yellow/red phaeomelanin (Figure 2.24). Eumelanin has photoprotective qualities via its ability to absorb and scatter ultraviolet (UV) radiation and scavenge reactive oxygen species. In contrast, phaeomelanin is photosensitising and produces reactive oxygen species when exposed to UVA. After melanin is synthesised, pigment-containing melanosomes are transported to keratinocytes, internalised and trafficked to perinuclear locations where they can absorb UV light and protect keratinocyte nuclei from UV-associated radiation damage [3]. One of the critical transcription factors



(a) (b)

Figure 2.22 High-magnification views of dermal mast cell granules. (a) Typical scroll-like configuration of lamellae, some of which show a cross-banding of regular periodicity. (b) The substructure of this granule is a highly organised lattice (arrow). Courtesy of Professor R. A. J. Eady, St John's Institute of Dermatology, King's College London, UK.