Skin Barrier Immunity and Ageing

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Abbreviations:

APC – Antigen presenting cells

DAMPs - Danger associated molecular patterns

DC – Dendritic cells

DETCs - Dendritic epidermal γδ T cells

DTH - Delayed-type hypersensitivity

ILC - Innate Lymphoid cell

MMP - Matrix metalloproteinases

LCs - Langerhans cells

TLR – Toll-like receptor

T_{cm} - central memory T cells

TNF - Tumour Necrosis Factor

T_{rm} – T resident memory cells

Tregs - T regulatory cells

UV - Ultraviolet

VZV – Varicella Zoster Virus

Abstract:

The skin is the outermost layer of the body with an extensive surface area of approximately 1.8 m^2, is the first line of defence against a multitude of external pathogens and environmental insults. The skin also has important homeostatic functions such as reducing water loss and contributing to thermoregulation of the body. The structure of the skin and cellular composition work in harmony to prevent infection, deal with physical and chemical challenges from the outside World.

In this review we discuss how the structural cells such as keratinocytes, fibroblasts and adipocytes contribute to barrier immunity. We also discuss specialised immune cells that are resident in steady-state skin such as mononuclear phagocytes such as Langerhans cells, dermal macrophages and dermal dendritic cells in addition to the resident memory T cells.

Ageing results in increase in skin infections and increased cancer incidence. As we age the skin structure changes with thinning of the epidermis and dermis, increased water loss and fragmented collagen and elastin. In addition the skin immune composition changes with reduced Langerhans cells, decreased antigen-specific immunity and increased regulatory populations such as Foxp3+ Tregs. Together, these alterations result in decreased barrier immunity in the elderly explain in part their increased susceptible to cancer and infections.

1. Skin Barrier:

The skin is the outermost layer of the body with an extensive surface area of approximately 1.8 m^2, is the first line of defence against a multitude of external pathogens. The skin consists of three layers: above is the epidermis, a thin layer (approximately 0.1mm thick) of stratified squamous epithelium, composed of four strata of keratinocytes in progressive stages of differentiation. The stratified epithelium provides a watertight barrier from the external environment and prevents excessive water loss from the body. The epidermis is mainly composed of keratinocytes, however there are also melanocytes are present which provide a barrier in the skin from Ultraviolet (UV) radiation via expression of melanin. The epidermis does not have a blood supply of its own, but instead is nourished from blood vessels below. The second layer is the dermis, a thicker layer (up to 3-4 mm depending on body site) which has a relatively low cell volume as compared to the epidermis. The dermis predominantly consists of the extracellular matrix, such as collagen which is made by fibroblasts. In addition to the extracellular matrix dermis contains structures such as blood vessels, lymphatics, nerves, sweat glands and pilosebaceous units. The deepest layer of the skin is the subcutaneous layer, which consists of subcutaneous fat and connective tissue (1).

2. Skin barrier immunity:

The skin is a complex organ which carries out numerous functions contributing to its barrier immunity function – the skin structure and stromal and immune cell composition can be seen in Figure 1.

Antimicrobial peptides and lipids are secreted onto the cell surface to control bacterial growth. These include dermcidin, which is secreted in human sweat and has broad anti-microbial activity against a range of pathogenic bacteria, and its antimicrobial activity is not affected by the low pH value and high salt concentrations of human sweat (2). Sebum is made by sebaceous glands found independently of or near hair follicles, within the sebum are antimicrobial lipids, such as lauric acid and sapienic acid, which play an important role in controlling pathogenic organisms (3).

However, the skin is not a sterile site, and there is extensive research showing the role the skin microbiota plays in immunity by restricting the growth of pathogenic bacteria (4). Commensal bacteria have been shown to produce an antimicrobial peptide which synergizes with the human antimicrobial peptide LL37, which together kill the pathogenic bacterium *Staphylococcus aureus (5)*. However, insults and pathogens are in the majority controlled and prevented entry due to structure and barrier immunity in the skin.

2.1 Skin resident stromal cells:

Keratinocytes are the main component of the epidermis. They express Toll-like receptors (TLRs), which are crucial pathogen pattern recognition receptors that when triggered lead to the production of inflammatory cytokine and initiation of an immune response (6). Keratinocytes have been shown to constitutively express TLR1, 2, 3, 5, 6 and 10 (7, 8). They also have the ability to sense wound damage and produce inflammatory cytokines and chemokines such as IL-1β, IL-8 and CCL20 to recruit leukocytes to the site of damage (9).

Keratinocytes express a raft of antimicrobial peptides that control bacterial growth including adrenomedullin and β-defensins (10, 11). β-defensin-1 is constitutively expressed by human keratinocytes and β-defensin 2 and 4 are upregulated upon inflammatory challenge (11-13). Keratinocytes can express the antimicrobial peptide Cathelicidin upon stimulation and can store Cathelicidin in cytoplasmic granules until needed (14, 15). Keratinocytes also express RNase 7 constitutively, which is a very potent antimicrobial ribonuclease, and upon inflammatory or bacterial challenge there is further increased expression (16).

More recently, it has been proposed that Keratinocytes a have the ability to process and present antigen to $CD4^+$ and $CD8^+$ T cells, initiating an adaptive immune response (17). The keratinocytes are the key site for the first step in the vitamin D metabolism pathway, as pro-vitamin D3 (7-dehydro-cholesterol) is metabolised into vitamin D3, as catalysed by UVB - as Vitamin D is an important component of a functioning immune system the metabolism of this at the skin site contributes to barrier immunity (18).

Dermal fibroblasts are the structural cells of the dermis, their primary function is to secrete extracellular matrix components such as pro-collagen. Fibroblast express the full range of TLRs, at a higher level than keratinocytes, demonstrating their important role in the detection of pathogens (19). *In vitro* studies have shown that dermal fibroblasts can have differing roles in immunity, indeed TLR4 signalling results in production of inflammatory cytokines such as IL-6, IL-8 and the monocyte chemoattractant CCL2 (20). Whilst conversely fibroblasts have been shown to suppress T cell proliferation via IDO production, and skew the T cells to produce immunoregulatory cytokines such as IL-10 (21).

The subcutaneous layer of the skin is predominantly composed of adipocytes – their primary function is to be a repository of energy which responds to hypothermia by producing heat. More recent work has identified the important role of adipocytes in barrier immunity as a significant source of antimicrobial peptides. In response to infection, for example to *Staphylococcus aureus*, dermal fibroblasts can differentiate into adipocytes and produce the antimicrobial peptide cathelicidin (22).

2.2 Skin resident immune cells:

2.2.1 Mononuclear phagocytes

Within the epidermis there is a population of mononuclear phagocyte called Langerhans cells (LCs) – they have historically been believed to have been seeded at birth and repopulated locally maintaining a steady state population (23). However a recent study demonstrated, in the murine model of immune injury, that is a repopulation of LCs from peripheral monocytes to make up for the slow repopulations from mature LCs (24). LCs they are located at the interface with the external environment and as such are multifunctional, sentinels of the epidermis. LCs sample the environment via their extension and retraction of their dendrites between the keratinocytes in amoeba-like movement (25). They present antigen to T cells within the epidermis to initiate a local immune response and also have the capacity to migrate to the lymph node and initiate immune responses (26).

Within the dermis there is a more diverse population of mononuclear phagocytes with dermal dendritic cells (DC) and dermal macrophage populations. Dendritic cells are the sentinels of the immune system, they sample the microenvironment and either present antigen to the resident T cells or migrate through the lymphatics to the lymph node to initiate an immune response (27). Historical assessment of dermal DCs identified that they are more activated then their blood counterparts; dermal Dcs had increased expression of co-stimulatory receptors and were strong stimulators of T cell proliferation relative to their peripheral blood counterparts (28). |It has been identified that there are two main populations of dermal myeloid DCs; the CD1c+ DCs and the CD141+ DCs. CD141+ DCs are the cells responsible for cross-presenting antigens to CD8+ T cells and have homology to the mouse CD103+ DCs (29). Very few plasmacytoid DCs are observed in steady-state skin (30).

Macrophages are antigen presenting cells resident in the dermis and sense pathogens and damage and initiate an appropriate immune response. In addition to the immune function, macrophages maintain tissue homeostasis through increasing appropriate anti-inflammatory mechanisms, contribute to wound healing and heal nerves upon tissue injury (31, 32). Macrophages are thought to populate tissues early on but that studies have also shown that they are replenished by circulating monocytes (33). This data is supported by a study in humans which showed that CD14+ cells were a transient population of monocyte-derived macrophages (34). CD163 has been proposed to be a good marker for dermal macrophages, as it specifically identifies skin–specific macrophages which are not recently migrated monocytes (35).

Analysis of the location of these different mononuclear phagocyte populations in the dermis have shown that DCs can be found closer to the epidermis (around 0-20µm beneath the dermo-epidermal junction) and macrophages were located deeper in the skin (around 40-60µm beneath the dermo-epidermal junction) (36).

2.2.2 Other innate populations

In rodent and cattle skin a population of γδ T cells has been described called Dendritic epidermal γδ T cells (DETCs) - these cells are localised in the epidermis (37). DETCs express a limited T cell receptor repertoire and recognise danger associated molecular patterns (DAMPs) induced on damaged or dysregulated keratinocytes. In addition, DETCs have also been shown to play a role in maintaining keratinocyte homeostasis, as in the absence of DETCs there was increased keratinocyte apoptosis (37). However, DETCs have not been observed in human skin. Indeed, in human skin the predominant leukocyte population is αβ T cells, γδ T cells and NK cells were found in the skin but at very low frequencies (0.35% and 0.97% respectively) (38). Neutrophils are not present in steady-state skin – however upon sun exposure there is an infiltration of neutrophils which contribute to sun burn and photo-ageing (39).

Innate Lymphoid cells (ILC) are a relatively recently described immune cell population and their function in the skin is still under investigation. In steady-state human skin there are sparse number of ILCs, and those cells that are present tend to be ILC1 and ILC3 - only upon an inflammatory response are ILC populations observed in significant numbers (40). In atopic dermatitis there is an influx of ILC2s and in psoriatic plaques there is ILC1 and ILC3 populations (40, 41).

The dermis also contains mast cells, of which there are between 77-108 cells/mm^2 (42). Mast cells contain granules with pre-formed inflammatory mediators such as histamine that are released when receptors are crosslink, contributing to local inflammatory response. Mast cells also play an important role in allergic reactions and associated itching and rash.

2.2.3 T cells

Skin T resident memory cells (T_{rm}) are non-circulating T cells present in the skin who maintain immune surveillance and are crucial for initiating a robust immune responses at times of infection (43-45). In steady-state skin there are around $1x10^6$ T cells/cm^2 suggesting that in an average person there is around $2x10^{10}$ T cells present in the whole skin (46). The majority (80-90%) of T cell found in the skin are T_{rm} the remaining T cells are

recirculating T cells (47). Cutaneous T_{rm} are generated after exposure to antigen and provide memory at the site of initial exposure - T_{rm} are more potent effector cells as compared to circulating T cells (47). Of the CD3+ T_{rms} present in the skin the ratio of $CD4^+$ to $CD8^+$ T cells was found to be approximately 3:1 in human epidermis and 6:1 in dermis (47).

The most commonly used markers to define T_{rm} cells are cell surface expression of CD69 and CD103 (48). T cell increase CD69 expression in response to antigen exposure or Type I Interferon (IFN) signalling, and this blocks T cell egress from the skin via inhibiting the sphingosine-1-phosphate receptor function (49, 50). CD103 is an integrin that binds to E-cadherin, it has been shown to be a marker more for $CD8^+$ T_{rm} present in the epidermis (47, 48). CD103 expression in the epidermis is believed to be due in part to the expression of E-cadherin on the keratinocytes which is important for retention of these cells in the epidermis (51).

In addition to CD69 and CD103, CCR8 has been proposed to be a T_{rm} marker (52, 53). The sole ligand for CCR8 is CCL1, which is predominantly expressed by CD1a+ Langerhans cells (52). The epidermis and in particular keratinocytes have been shown to play a role in upregulating CCR8 on naïve T cells in the skin and generating T_{rm} cells, through production of Vitamin D3 and Prostaglandin E2 (53, 54).

$CD4^+$ FoxP3 T regulatory cells (Tregs) are an important regulatory cell type that play an important role in immune and tissue homeostasis (55). $Foxp3^+$ Tregs with a memory skin-resident phenotype have been observed in the dermis and in particular in steady state conditions can be found located closely to hair follicles (56). The short-chain fatty acid Sodium Butyrate, which is a bacterial metabolite produced by skin commensals, can increase Foxp3 expression in non-Tregs driving an increase $Foxp3^+$ Tregs (57). In addition, UVB light has been shown to increase number of $Foxp3^+$ Tregs via facilitating the proliferation of thymically derived $Foxp3^+$ Tregs (58). This effect of UVB could be in part due to the production of Vitamin D3 which can drive Foxp3+ Treg proliferation *in vitro* (59). This function of Sodium Butyrate leads to immune tolerance to the skin commensal bacteria. Indeed it is believed $Foxp3^+$ Tregs accumulate around the hair follicle due to entry of commensal bacteria to newly formed hair follicles during neonatal skin development (60).

2.3 Ageing and skin structure:

As we age our skin structure changes (Figure 2). The epidermal layer is thinner due to keratinocyte atrophy observed in older skin (61). This leads to increased trans-epidermal water loss in elderly individuals resulting in increased skin dryness (62). The extracellular matrix components collagen and elastin which provide tensile strength and elasticity

respectively, are substantially changed with age. The total amount of collagen has been shown to be reduced with age (63). However there is also increased collagen fragmentation which is believed to be due to increased Matrix metalloproteinase (MMP) expression in older skin (64). Elastin is an inert protein which is formed during early development and is not replenished, therefore any changes to elastin which occur over a life-time tend to be permanent (65). MMPs, in particular MMP-1, -3 and -9 target elastin for fragmentation (65), resulting in reduced skin elasticity and the classical sign of skin ageing, wrinkling.

Dermal fibroblasts contribute to age-associated dermal thinning as they are reduced in size (66). In addition dermal fibroblasts from elderly individuals make less pro-collagen and increase expression of MMP-1 contributing to increase collagen fragmentation (66-68). Other changes in the skin which are observed with age are reduced sweat and sebum production (69). Finally, there is a thinning of the adipose tissue observed with age due to a reduction in white adipose tissue – subsequent anti-microbial protection (by the dermal fat) in response to infection is significantly decreased. This reduction in adipocytes is believed to be due in part to the inability of fibroblasts to convert to adipose tissue (70).

Changes in skin structure with age are dependent upon lifestyle choices and environment challenges, as UVB exposure and the use of sun screen, smoking and environmental pollution (71, 72). Collectively these changes render older people more susceptible to mechanical injury, alter the skin microbiome and have important implications for skin barrier immunity.

2.4 Immunological changes in the skin with age:

The decrease in cutaneous immune function has been well documented in older humans. A variety of bacterial infections are more common in the elderly, including cellulitis (in particular of the lower legs), erysipelas, necrotizing fasciitis, folliculitis, impetigo, folliculitis, and furunculosis (73). *Staphylococcal aureus* and B-haemolytic *streptococci* are the most common skin pathogens in the elderly, although other bacterial infections caused by *Pseudomonas spp* and *Klebsiella spp* are also elevated in older individuals (74). The prevalence of skin colonisation by Proteus mirabilis and Pseudomonas aeruginosa in the over 65-year-old population is increased by about 25% compared with younger individuals (74). Fungal infections (such as *Candida*) and viral infections such as shingles, Herpes Simplex Virus-1 and Human Papilloma Virus are also more common in the elderly (74, 75).

Non-melanoma skin cancer, including basal cell and squamous cell carcinoma, is more commonly diagnosed in persons older than 70 years. The highest incidence of malignant melanoma and melanoma is in individuals aged 65 years and older (75-78).

Together these observations provide strong evidence for age-dependant changes in the skin barrier immunity. Although changes in peripheral immune cell populations have been well described (as reviewed previously (79-81)), we have focussed on skin-specific immunological differences with age (Figure 3).

2.4.1 Mononuclear phagocytes:

Langerhans cells are reduced in number in the elderly. In addition LCs from older donors have reduced migratory capacity to the lymph node (82). Using an *ex vivo* epidermal model Pilkington *et al* have shown that lower levels of IL-1β observed in elderly skin, result in reduced migration of the LCs to the cytokine gradient – demonstrating that the skin microenvironment plays a detrimental role (83). The specific source of IL1β in the skin remains controversial, and both keratinocytes and LCs themselves have been proposed as the primary source. In addition, LC from aged skin, express less human β-defensin 3, an important antimicrobial peptide for response to infection (84).

The number and phenotype of dermal DCs is comparable between young and old skin (81). However, dermal DCs from aged skin appear to be functionally impaired in terms of migration, phagocytosis and ability to stimulate T cells in a mouse B16 melanoma model (85). The effect off age on macrophage function is still contentious - some studies demonstrate reduced TLR expression and TLR-induced cytokine production (86). Whilst other studies show that there is increased inflammatory cytokine production after TLR ligation (87). However, there is limited data on the effect of age on dermal macrophage populations. We have shown that when CD163+ macrophages produce less TNFα in antigen challenged skin, however upon removal of the macrophages from the skin environment they produce similar amounts of pro-inflammatory cytokine in response to TLR ligands (82). Thus, suggesting that it is the skin environment itself which is altered with age rather than intrinsic dysfunction of macrophages.

2.4.2 T cells:

Repeated antigen stimulation throughout life can have significant effects on human antigen specific T cells including the induction of exhaustion and senescence. Functional exhaustion of T cells is characterised by the loss of functional activity, increase in inhibitory receptor expression (such as PD-1). It is a mechanism necessary for limiting the magnitude of the effector T cells response but it also contributes to the functional decline in the adaptive immunity with age. Senescence, a loss of replicative capacity, is often induced by repeated

stimulation, and is primarily induced through the process of telomere erosion. While the age –related changes in the circulating T cell pool have been well characterised and reviewed extensively (79), the age related changes in the skin resident T cell population have not been extensively studied. The differences in the regulation of senescence and the importance of telomere shortening between mouse and human T cells should also be taken into account when extrapolating from mouse models (88).

Tissue resident CD8+ T cells have recently been shown to promote a long lasting state of equilibrium between melanoma and the immune system (89). Depletion of these T_{rm} demonstrated that they actively suppress tumour progression (89). How anti-tumour surveillance and control by skin resident T_{rm} is affected by age and age–related changes within in the CD8 population has not been studied. It is known that skin resident T_{rm} cells are vital to clear skin infections (90-92), therefore defects in T_{rm} cells may explain the increased incidence of infection seen in the elderly. We and other have shown that there is decreased Delayed-type hypersensitivity (DTH) responses to recall antigens such as *Candida* or Varicella Zoster Virus (VZV) (75-78) in older adults due to a reduced infiltration of T cells at the site of antigen challenge. Our group has shown that the function of skin derived CD4+ T cells was not impaired with age in response to both mitogen and antigen-specific stimulation *ex vivo* (93) although the skin residency markers were not used for cell isolation. Interestingly old skin actually had a higher proportion of VZV-specific T cells compared to young- possibly suggesting accumulation over a lifetime of subclinical reactivation (94). There was however an increase in PD-1 expression on both CD4 and CD8 T cells in old individuals as compared to young skin, this data suggests that older T cells are more susceptible to inhibition via PDL-1/PD-1 signalling (93).

2.4.3 Foxp3$^+$ Tregs

The proportion of regulatory cells in normal skin is increased in older mice and human (95, 96). Foxp3$^+$ Tregs accumulate during a cutaneous immune response.In those people who had the highest proportion of Foxp3$^+$ Tregs they had the worst DTH response to VZV recall antigen – showing that Foxp3$^+$ Tregs in the skin can interfere with antigen-specific immunity (97). Indeed, in a mouse model of melanoma, Tregs can suppress very early stages of the inflammatory response to antigen challenge (98). It is known that there is an increase in Foxp3$^+$ Treg numbers in the skin in cancers such as melanoma and basal cell carcinoma (99-101). In human squamous cell carcinoma of the skin, 50% of cells have a Foxp3$^+$ Treg phenotype, reduction of Foxp3+ Treg percentage in these patients and their function led to clinical improvement (102). The reasons Foxp3+ Treg numbers are increased in older skin

are not clear. It has been shown that UVB irradiation can lead to the induction of Foxp3+ Tregs and that these Foxp3+ Tregs suppress other immune cells through the production of IL-10 (58, 103). It is also tempting to postulate that Foxp3+ Tregs could be induced or accumulate as an attempt to the immune system to control unwanted low grade inflammation which accompanies ageing.

2.4.4 Inflamm-ageing and senescence in the skin

Chronic low grade inflammation termed inflamm-ageing is characterised by high serum CRP (104). Inflamm-ageing is known to negatively impact on immunity as in older people who had elevated IL-1β they had increased risk of morbidity and mortality (105). It has been postulated that innate immune cells such as macrophages are a contributor to the inflamma-ageing phenotype, as due to changes in tissue structure –such as skin thinning – they are exposed to more bacteria which leads to chronic activation and subsequent inflammatory cytokine production, such as seen with increased gut permeability in an aged mouse model (106).

Another contributor to inflamm-ageing especially in the skin is UV damage. Repeated exposure to UVB, as would be the case in old skin, leads to the accumulation of macrophages and increase in ROS and MMP, and subsequent damage to extracellular matrix. Inappropriate complement activation may also be caused by the increase in oxidative stress and accumulation of damaged cells, in line with observations in atherosclerosis (107). This complement activation and chronic activation of macrophages could contribute to inflamm-ageing in the skin. Another contributor to increase inflammation in the old is the accumulation of senescent cells, senescence is defined as irreversible growth arrest. It is known that there is an accumulation of senescent dermal fibroblasts, as classically defined by p16 expression in the skin of old mice and humans (108-110). Senescent fibroblasts secrete a raft of inflammatory mediators such as IL-8, IL-6, TNFα and CCL2 (110). This production of inflammatory mediators from senescent cells is termed senescence associated secretory phenotype (SASP), which contribute to the low-grade inflammation observed older individuals (111). A recent paper has shown senescent dermal fibroblasts persist in the skin by evading recognition and killing from NK cells and CD8+ T cells, through increased expression of HLA-E (110). Other skin resident cell populations that have shown to be senescent include endothelial cells and melanocytes (112, 113). Although increased frequency and number of senescent T cells have been observed in the periphery (80), their contribution to the skin environment unknown and warrants further investigation.

How this inflammation directly negatively affects cutaneous immune responses is not clear. Our studies have shown that skin from older individuals have a propensity to mount an inappropriate response to saline injection which negatively correlates with antigen-specific cutaneous immunity (94). Furthermore, blocking inflammation using a p38-MAPKinase inhibitor, Losmapimod, reduced this non-specific inflammation while improving the ability of old individuals to respond to recall antigen challenge (94).

2.5 Concluding remarks:

The skin barrier immunity is comprised of stromal cells such as keratinocytes and adipocytes and immune cells such as Langerhans cells and T_{rm} working in concert to prevent pathogen entry and to deal with continuous physical and chemical assaults (challenges). With increasing life-span, it is important to understand how skin changes with age and the impact these changes have on barrier immunity. Clearly the skin environment is detrimental to a successful immune response in the skin of older people as removal of individual cells from the skin microenvironment results in restoration of immune function. Specifically which cells alter the ageing skin environment is unknown, certainly senescent cells such as fibroblasts will contribute greatly. However there is still more research required to understand fully which cells are responsible for the ageing skin microenvironment and cells types such as keratinocytes, endothelium and adipocytes warrant further investigation. Better understanding of inhibitory and inflammatory mechanisms that operate in older skin is crucial for developing of new strategies to combat infections and cancer

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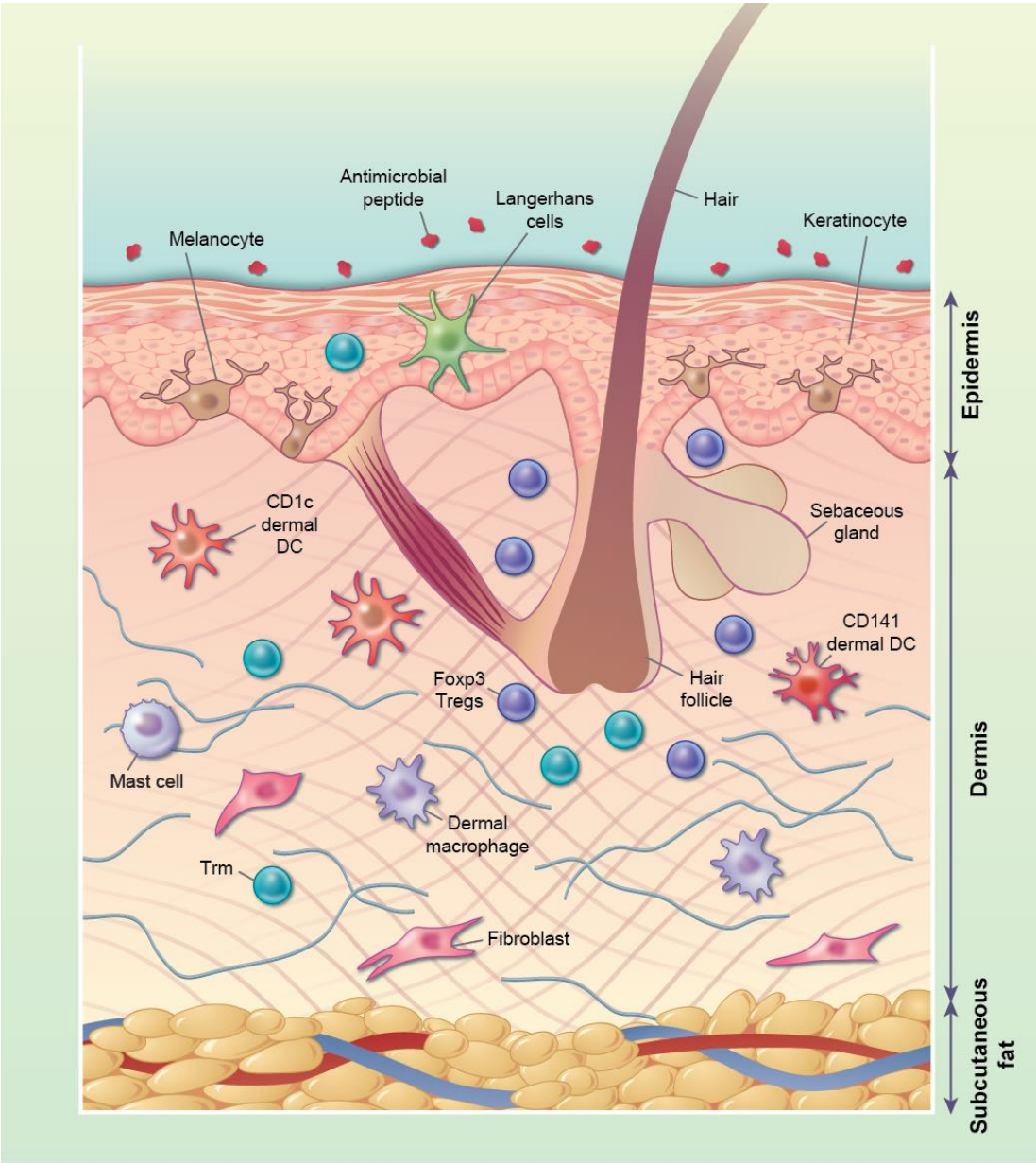
Antimicrobial peptide
Langerhans cells
Hair
Melanocyte
Keratinocyte
Epidermis
CD1c dermal DC
Sebaceous gland
CD141 dermal DC
Foxp3 Tregs
Hair follicle
Dermis
Mast cell
Dermal macrophage
Trm
Fibroblast
Subcutaneous fat

Figure 1: Human skin barrier immunity

Diagrammatic representation of human skin barrier immunity. The surface of the skin is covered in antimicrobial peptides and lipids, some of which originate from the sebaceous gland located near the hair follicle. The epidermis consists of keratinocytes forming stratified corneum, with melanocytes interspersed. Langerhans cells and T resident memory cells (T_{rm}) can also be found in the epidermis. The dermis has a more diverse collection of cells including structural cells such as fibroblasts, and immune cells such as dermal dendritic cells (DCs) and macrophages, CD4+ and CD8+ T_{rm}, mast cells and Foxp3+ T regulatory cells (Tregs) which are often located near the hair follicle. The final layer of the skin is the subcutaneous fat which is primarily composed of adipocytes.

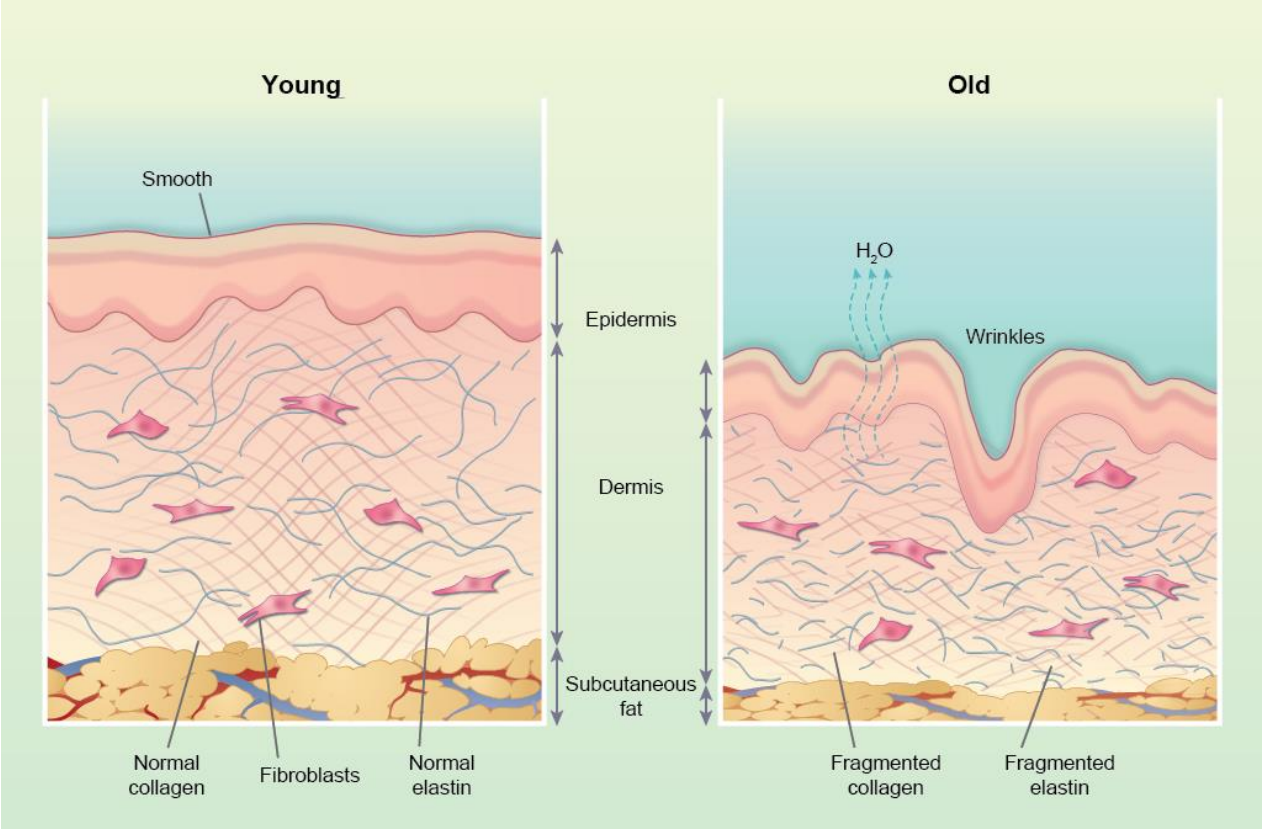

Figure 2: Structural changes in human skin with age.

Young skin structure (left) and compared to older skin structure (right). Older skin has fragmented elastin and collagen, increase water (H_2O) loss which leads to skin dryness and increased wrinkles. In addition, the skin is thinner with all three layers being less thick then the younger counter part.

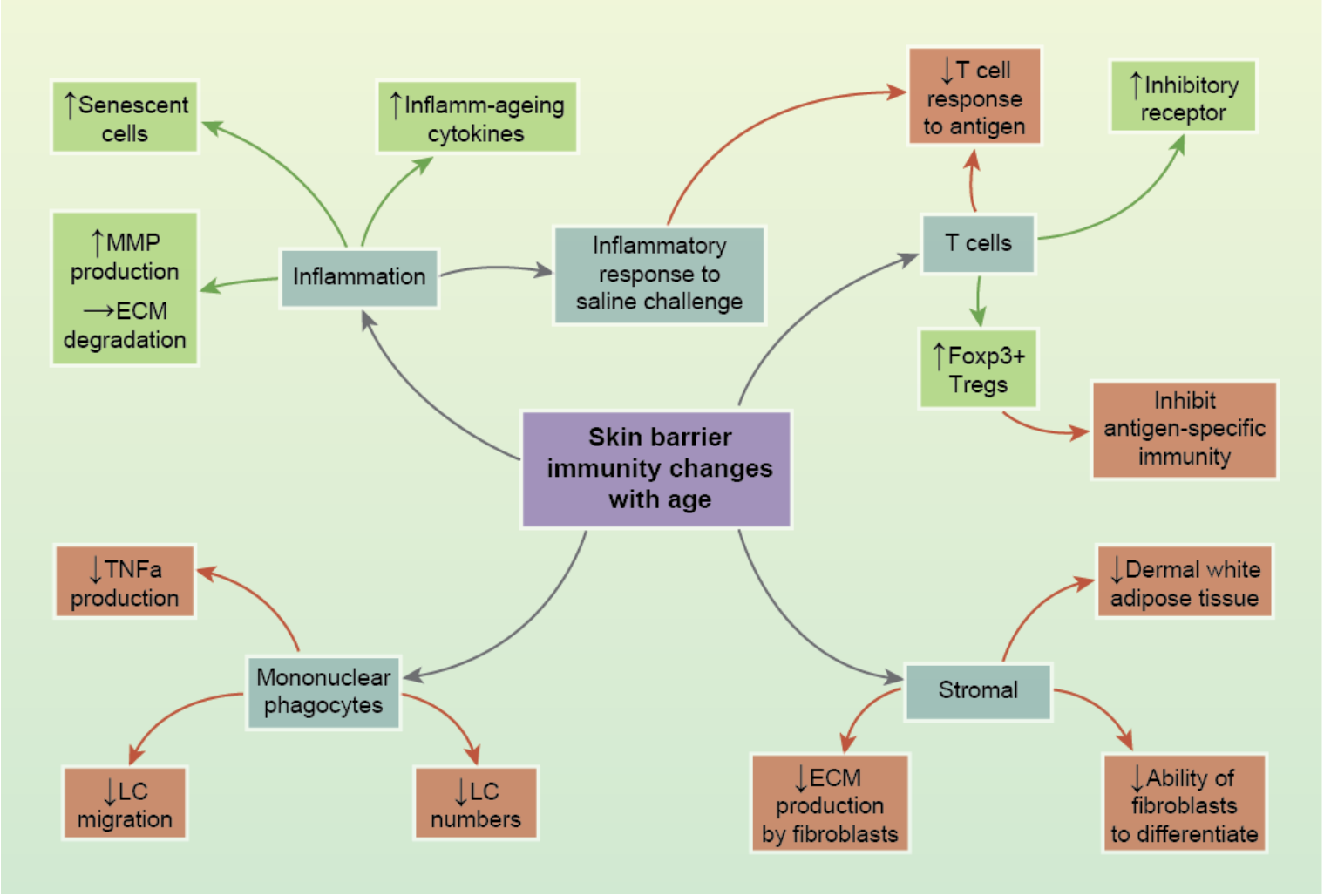

Figure 3: Skin barrier immunity changes with age. Schematic showing the effect of age on skin resident populations. Negative/inhibitory effects are shown in red and positive/enhancing effects shown in green. ECM = Extracellular matrix; LC = Langerhans cell; MMP = Matrix metalloproteinases; Treg = T regulatory cells