


## VIEWPOINT

# Sonic Hedgehog signalling in the regulation of barrier tissue homeostasis and inflammation

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Epithelial barrier tissues such as the skin and airway form an essential interface between the mammalian host and its external environment. These physical barriers are crucial to prevent damage and disease from environmental insults and allergens. Failure to maintain barrier function against such risks can lead to severe inflammatory disorders, including atopic dermatitis and asthma. Here, we discuss the role of the morphogen Sonic Hedgehog in postnatal skin and lung and the impact of Shh signalling on repair, inflammation, and atopic disease in these tissues.

## Introduction

The mucosal and barrier sites such as the skin, gastrointestinal tract, and airway form an essential interface between the mammalian host and the external environment. These physical barriers are crucial to prevent damage and disease from environmental insults and allergens. Failure to maintain barrier function against such risks can lead to severe inflammatory disorders, including atopic dermatitis, asthma, gastric inflammation and inflammatory bowel disease. Maintenance of

barrier defence relies on the complex interplay of innate and adaptive immune cell responses together with physical barrier function intrinsic to the tissue, which together are essential for restoration of tissue homeostasis following damage, disease, and environmental challenges.

The Hedgehog (HH) family of intercellular signalling proteins activate a highly conserved signalling pathway that plays an essential role in developmental patterning during embryogenesis and is also important

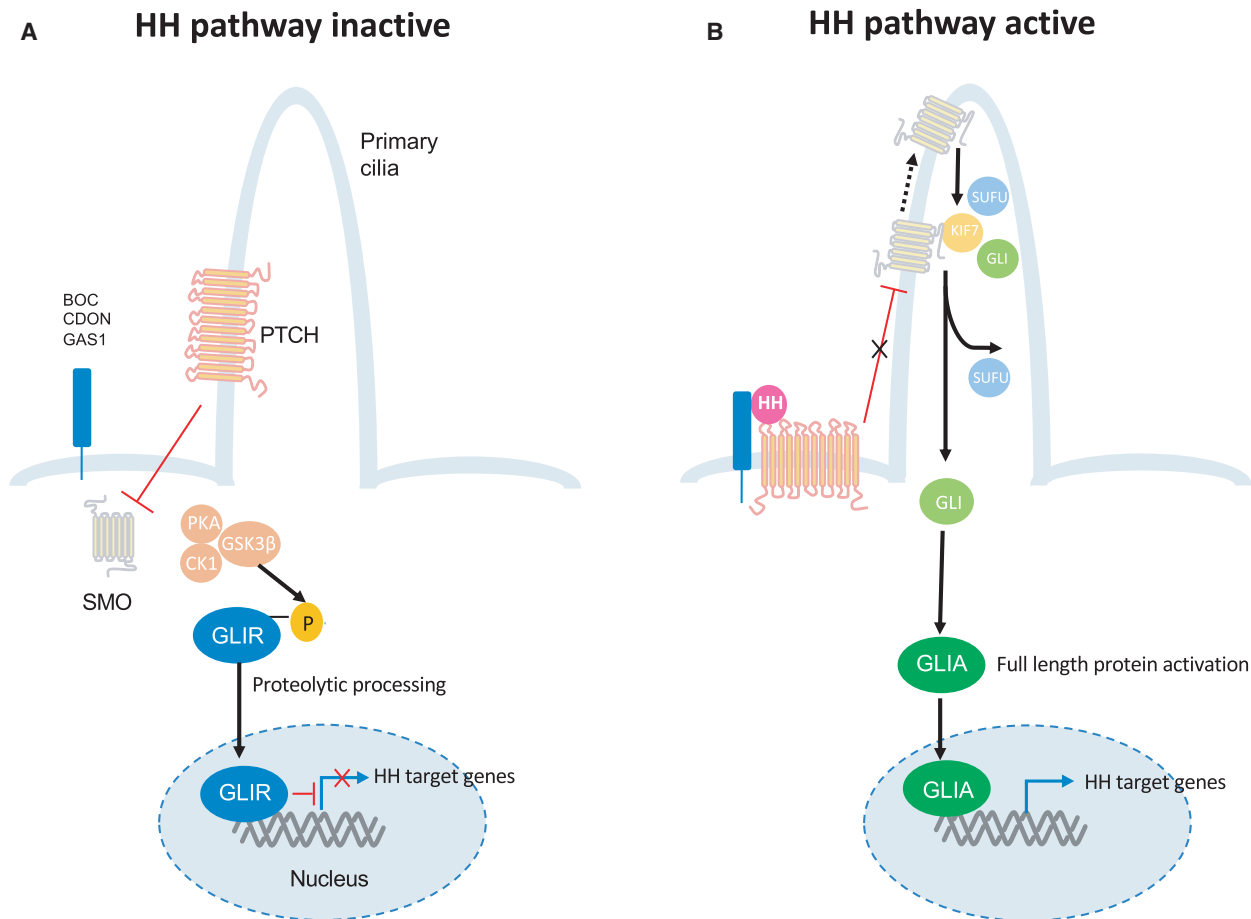
## Abbreviations

AD, atopic dermatitis; BALF, bronchoalveolar lavage fluid; BOC, brother of CDO; CDO, CAM-related/downregulated by oncogenes; CTL, cytotoxic T lymphocytes; DHH, desert hedgehog; GAS1, growth arrest-specific 1; GLI, glioma-associated oncogene; GSK3 $\beta$ , glycogen synthase kinase 3 $\beta$ ; GWAS, genome-wide association studies; HH/Hh, hedgehog; HHIP/Hhip, Hedgehog interacting protein; IFITM, interferon inducible transmembrane; IHH/lhh, indian hedgehog; IPF, idiopathic pulmonary fibrosis; LAP, latency associated peptide; PKA, protein kinase A; PTCH1/Ptch1, patched1; PTCH2, patched2; SHH/Shh, sonic hedgehog; SMO/Smo, smoothened; SUFU, suppressor of fused; Tgf $\beta$ , transforming growth factor; Th, T helper; Treg, T regulatory cells.

in homeostasis and regeneration of adult tissues [1,2]. The three mammalian HH proteins, Sonic hedgehog (SHH), Indian hedgehog (IHH), and Desert hedgehog (DHH) share a common signalling pathway, which is initiated by binding of HH proteins to their receptor, the 12-transmembrane protein Patched1 (PTCH1), thereby allowing the 7-transmembrane G protein-coupled receptor smoothed (SMO) to be phosphorylated and enter the primary cilium to trigger the downstream events (Fig. 1). In addition to PTCH1, HH proteins also interact with Patched2 (PTCH2) and cell-surface molecules CAM-related/downregulated by oncogenes (CDO), brother of CDO (BOC) and growth arrest-specific 1 (GAS1) in vertebrates, which function as co-receptors. The accumulation and activation of SMO leads to the translocation of suppressor of fused (SUFU) to the primary cilium, and the kinesin protein

KIF7 is also recruited to the cilium tip, allowing dissociation of the glioma-associated oncogene (GLI) family transcription factors from SUFU, which prevents the phosphorylation of the GLI protein and leads to translocation of full-length and active GLI into the nucleus, where they activate and promote HH target gene transcription [1,3–5]. In addition to this canonical HH signalling pathway, which involves movement of components of the pathway in the primary cilium, several different non-canonical forms of HH pathway activation have been described, including SMO-independent signalling and HH pathway activation in cells that lack primary cilia such as lymphocytes [6,7].

In the absence of HH ligands, SMO activity is inhibited by PTCH1. The C-terminal activation domain of the GLI transcription factors then undergo phosphorylation by protein kinase A (PKA), casein



**Fig. 1.** The Canonical Hedgehog signalling pathway. (A) In the absence of HH, PTCH1 accumulates in the primary cilium and suppresses SMO activation. The GLI transcription factors are phosphorylated by PKA, CK1 and GSK3 $\beta$ , leading to their proteolytic cleavage to generate the repressor forms of GLI (GLIR), which inhibit transcription of HH target genes. (B) When HH ligands bind to PTCH1, BOC, CDON and GAS1, SMO is released from PTCH1 inhibition. This allows for dissociation of GLI from SUFU and KIF7, leading to translocation of full-length activator forms of GLI (GLIA) into the nucleus to promote HH target gene transcription.

kinase 1 $\alpha$  (CK1), and glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), which results in their proteolytic cleavage to generate the repressor forms of GLI. This results in suppression of transcription of HH target genes in the nucleus [1,8,9].

In mammals, three downstream GLI transcription factors have been identified, GLI1, GLI2 and GLI3 each with specialized functions. GLI1 lacks the N-terminal repressor domain and thus functions exclusively as a transcriptional activator and is itself a target gene of HH signalling, whereas GLI2 and GLI3 can be processed to function as transcriptional activators or transcriptional repressors, with GLI2 acting mainly as an activator and Gli3 acting predominantly as a repressor, in the presence or absence of the pathway respectively [10–14].

While the best-characterized roles for HH signalling are its morphogenic functions in patterning during embryogenesis, a growing body of research has emerged indicating that HH signalling is involved in regulation of haematopoiesis, the immune system, and inflammation [15–22].

Hh pathway components are expressed in the thymus and signal to regulate differentiation and proliferation throughout multiple stages of T-cell development. In mouse studies, Shh and Ihh, secreted from thymic epithelial cells, have been shown to promote expansion, survival and differentiation of the earliest T-cell progenitors [23–25]. During  $\beta$ -selection, Shh and Ihh inhibit pre-TCR signalling and negatively regulate pre-TCR induced differentiation from DN to DP cell [25–27]. Following TCR $\alpha$  gene arrangement, Hh signalling negatively regulates the DP to SP transition, reducing the TCR-signal strength in developing thymocytes during TCR repertoire selection and influencing CD4/CD8 and  $\gamma\delta$ -lineage decisions [19,22,28–34].

In addition to T-cell development, Hedgehog proteins produced by peripheral tissues influence T-cell fate and function, including differentiation of CD4 T helper 2 (Th2) cells and effector function of CD8 cytotoxic T lymphocytes (CTL) [35,36]. Hedgehog signalling components are expressed in naïve CD4 and CD8 T-cells, and Gli2-dependent transcription can inhibit mouse CD4 and CD8 T-cell proliferation and activation in response to TCR and CD28 ligation [7,29,33,35–37]. Treatment with recombinant SHH also promotes Th2 differentiation in naive human CD4 T-cells, and attenuation of HH signal transduction reduces expression of the Th2 cytokine, IL-4, and Th2 key regulator GATA3 [37]. In CD8 T-cells, Ptch, Gli1 and Ihh were upregulated after TCR activation, and, using pharmacological Smo-inhibitors and mice with conditional deletion of Smo from the hematopoietic

system, Smo was shown to be required for efficient CTL-mediated killing, suggesting that Hh signalling promotes CD8 effector function [36].

Hedgehog signalling pathway components are expressed within various epithelial tissues, including the thymus, gastrointestinal tract, liver, airway, and skin epithelial cells, and it plays a crucial role in development and postnatal homeostasis of these tissues, whereas dysregulated Hedgehog pathway activation in epithelial tissues leads to several cancers [19,30,38–49]. However, in epithelial tissues, Hedgehog proteins also signal to resident and recruited immune and inflammatory cells, so that changes in their levels of expression on tissue damage may influence immune regulation, inflammation, and immune surveillance against tumours [35,39,50–53]. In this review, we discuss the role of Shh signalling in the epithelial barrier tissues skin and lung in inflammation and resolution of inflammation and homeostasis.

## Skin

Skin is the largest organ, which forms a barrier that isolates the body from the external environment and acts as the first line of defence against pathogens. Skin comprises the epidermis, the external layer in direct contact with the environment, and the inner dermis. Hair follicles and sebaceous and sweat glands are found in the epidermis, while the bulge is found in the lower part of the epidermis and is rich in stem cells. The skin barrier is formed by keratinocytes located in the epidermis and immune cells, which are either resident or recruited to the skin upon encounter with pathogens, which together maintain skin homeostasis. Antigen-presenting cells, called Langerhans cells, CD8-resident memory cells and dendritic epidermal  $\gamma\delta$ T-cells are present in the epidermis, while the dermis contains other immune cells, including macrophages, mast cells, neutrophils, innate lymphoid cells,  $\alpha\beta$ T-cells, and  $\gamma\delta$ T-cells, (reviewed in [54]).

Hh signalling is required for skin development. In the murine skin, expression of Hh signalling components including Ptch1, Smo, Gli1 and Gli2 are detected on day E15.5 in both the epidermis and dermis, while Shh and Ptch2 expression are solely detected in epidermis [55,56]. Shh is not required for initial stages of skin development, but it is involved later in hair follicle and dermal papilla development [56–60]. Loss of Shh is linked with decreased follicular development, interrupted keratinocyte proliferation, and impaired maturation of dermal papilla [59,61].

Although Hh expression is high during development, its expression in adult skin tissue is sustained at lower

levels. These lower levels of expression are tightly linked with the maintenance of skin homeostasis (Fig. 2), and aberrant Hh pathway activation results in skin cancer [49,62]. Shh, the main ligand of the Hh pathway expressed in postnatal skin, is detected in some populations of progenitor and stem cells [63] and is necessary for hair growth, renewal capacity of bulge stem cells [64], and balanced skin regeneration via proliferation of dermal fibroblasts [65].

The role of Shh in skin development and homeostasis is well known, but only recently studies have started to address the impact of Hh signalling in skin on immune responses. Skin inflammation is related to impaired skin barrier function [66,67]. Upon skin injury Shh is upregulated in the skin epithelium [39,68,69] and hair follicles [70], while *Gli1* and *Ptch1* are also expressed in the skin epithelium and dermis and in hair follicles close to the wounds [69,71]. Shh regulates keratinocyte proliferation rendering it responsible for maintenance of barrier integrity and skin repair [72,73]. In mouse postnatal cutaneous wound repair, it triggers embryonic stem cells to be included in the healing process, via promoting their proliferation and migration to the wounded area [72,74]. Shh pathway activation in epidermal cells of wounded skin results in the acquisition of hair follicle stem cell properties, leading to hair regeneration [69,70]. Furthermore, in a model of diabetes, Shh administration led to faster diabetic wound healing via regulation of the nitride oxide pathway [68].

Atopic dermatitis (AD) is a chronic inflammatory condition of the skin that coexists with other atopic diseases including allergic asthma. The loss of skin barrier integrity, which is observed in AD, is linked to keratinocyte apoptosis. Furthermore, primary cilia, which are essential for Hh signalling in epithelial cells, are increased in AD and other skin-related pathologies [75]. Although AD was initially described as a Th2 disease, independent studies have highlighted the importance of Th1 and Th17 responses in distinct phases of the disease [76,77]. In-vitro studies performed with both murine and human CD4 T-cells have shown that Shh controls Th1 and Th2 responses in mice and humans [35,37,78]. Recent findings from our group have shown that Shh expression in the skin epithelium reduced AD pathophysiology in a mouse model of atopic dermatitis [39]. Constitutive conditional transgenic expression of an activator form of *Gli2* in mouse T-cells resulted in decreased Th1, Th2, and Th17 cytokine production in skin, preventing the progression of AD [39]. Furthermore, we showed that Shh upregulation increased T regulatory cells (Treg) in skin and enhanced their immune suppressive function via

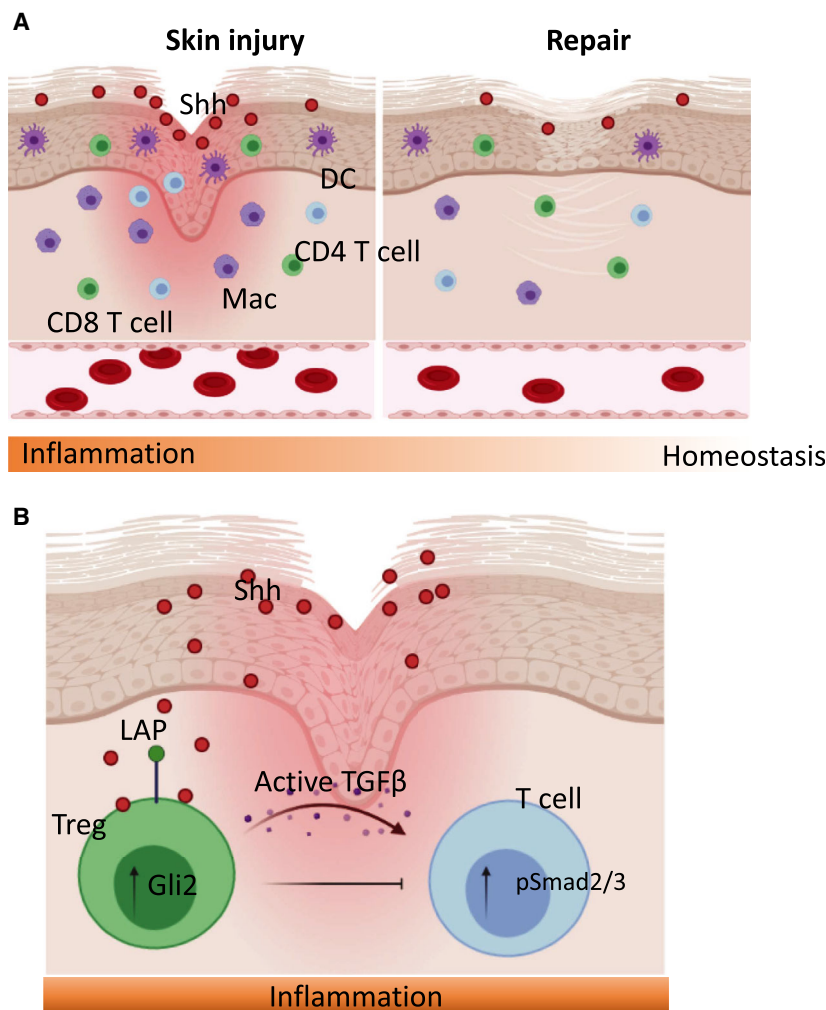
activating transforming growth factor  $\beta$  (Tgf $\beta$ ) signalling to skin T-cell populations [39]. Consistent with these data, Hh signalling favoured Treg accumulation and triggered skin IL10 and Tgf $\beta$  expression in a murine model of basal cell carcinoma, and inhibition of the pathway promoted the adaptive immune response to the tumour [53,79]. Additionally, inhibition of the pathway in a model of breast cancer led to a reduction in the Treg population while it favoured infiltration of Th1 and CD8 cytotoxic cells [80].

Together these data suggest that Shh expression in the skin epithelium is essential for the maintenance of skin homeostasis. In response to skin damage and barrier disruption, Shh is upregulated to constrain skin inflammation while it also triggers cutaneous repair (Fig. 2). Shh signalling to skin T-cells prevents skin inflammation and activation of the Hh signalling pathway augments skin Treg populations and promotes their immunosuppressive function promoting IL-10 and Tgf $\beta$  expression. However, it is important to highlight that dysregulated Shh pathway activation in skin can be oncogenic resulting in skin cancers such as basal skin carcinoma and that increased Shh signalling to immune cells may help skin tumours to evade immune surveillance.

## Lung

The lung possesses a highly dynamic epithelium with a broad spectrum of functions related to host defence, inflammation, immunity, and tissue remodelling. The Hh signalling pathway is involved in all these functions.

During lung embryonic development, Hh signalling plays essential roles in the early and late phases of mouse lung formation and branching morphogenesis by regulating proliferation and interaction of epithelial and mesenchymal cells [81]. In the embryonic phase of lung development, *Shh* is expressed at day E10.5 in the epithelial cells of the primary lung buds. During the pseudo-glandular stage Shh staining is highly detected in the distal rather than proximal epithelial cells of the primordial tubules. This proximal–distal expression pattern continues until day E16.5. As branch morphogenesis continues, Shh is localized in the non-ciliated specialized airway epithelial cells such as Clara cells, and its expression is sustained in the epithelium of bronchi, bronchioles, and terminal saccules. Then, *Shh* expression gradually decreases [82,83]. *Ptch1* is expressed at high levels at E11.5 in the mesenchyme adjacent to the terminal buds, with *Shh* expression in the surrounding epithelium [83,84]. Likewise, *Gli* genes are detected during the pseudoglandular stage in a



**Fig. 2.** Hedgehog signalling in skin repair and inflammation (A) Upon skin injury Shh expression is up-regulated in skin epithelium increasing Hh expression in skin. Shh signals to immune cells, including lymphocytes, and fibroblasts resulting in tissue repair. (B) In skin inflammation Hh signalling is responsible for the increased immune regulatory function mediated by regulatory T-cells. Shh secreted by skin epithelial cells signals to T-cells. That leads to improved Treg function via increased Gli2 activity and LAP expression and secretion of active Tgfβ. Active Tgfβ acts on effector T-cells increasing the expression of phosphorylated Smad2/3 rendering them less reactive and protects against skin inflammation.

distinct pattern of expression until all three genes are downregulated before birth [85].

Several mouse models genetically manipulated in Hh pathway components have confirmed the essential role of Hh during lung development. Shh-mutant mice have a clear phenotype with a presence of tracheo-oesophageal fistula, lung hypoplasia, and abnormal lung lobulation [86,87], whereas ectopic overexpression of *Shh* in the distal epithelium interrupts branching formation and increases epithelial and mesenchymal proliferation, leading to absence of functional alveoli and mice dying just after birth [83]. Absence of either *Gli2*, *Gli3*, or both genes also produces severe lung defects [85,88]. Hedgehog interacting protein (Hhip) is expressed in the mesenchyme at the early stages of embryonic lung development and absence of Hhip and increases Hh activation causing defective branching morphogenesis. The activity of the Hh pathway is reduced after postnatal alveolar development and in

adult tissues [89,90]. However, a recent study suggested that Hh signalling activity is retained in the adult lung and Shh signalling continues to function in a cell autonomous manner in mature lung to maintain quiescence/homeostasis by restricting mesenchymal proliferation [91].

The airway epithelium is constantly exposed to injury by pathogens, toxins, and trauma leading to a variety of inflammatory, immune, and defence responses. Following damage, a quick reparative proliferative program is required to restore lung homeostasis. It has been suggested that Hh signalling has a functional role in epithelium injury and repair by modulating proliferation and the immune response. Depletion of Clara cells (progenitor cells) by naphthalene exposure resulted in a transient peak of Shh and Gli1 expression 72h after depletion, which corresponded with the period of epithelial regeneration [44]. A similar increase in Hh signalling has been detected in a mouse



model of acute lung injury, during the period when expression of the pro-inflammatory cytokine TNF- $\alpha$  and the lung injury score decreases. Inhibition of Hh by cyclopamine-treatment upon lipopolysaccharide challenge increased *TNF- $\alpha$*  mRNA expression in lung tissue and injury score, also suggesting that Hh signalling may be involved in a protective mechanism to prevent additional damage via anti-inflammatory action [92]. Furthermore, overexpression of *Shh* in mouse airway epithelium leads to proliferation and lung tissue modifications comparable to those described during lung tissue repair and in injury models [93].

Hh signalling contributes to the development of chronic lung inflammatory diseases and tissue remodelling. SHH and PTCH1 are highly expressed in interstitial lung fibrosis and bronchiectasis [94]. In a mouse model of lung fibrosis, Shh and Ptch1 were up-regulated at sites of tissue remodelling and fibrosis. Ptch1 was also detected in infiltrating mononuclear cells [94], suggesting that Shh may be involved in immune and inflammatory infiltration to the airway during disease.

Genome-wide association studies (GWAS) and in vitro and in vivo models have implicated the negative regulators of the Hh pathway HHIP and PTCH1 in chronic obstructive pulmonary disease and emphysema [40,95–97]. SHH secretion levels are higher in bronchioalveolar lavage fluid (BALF) from idiopathic pulmonary fibrosis (IPF) patients than healthy controls [98]. Likewise, components of the Hh signalling pathway such as PTCH1, SMO, GLI1, and GLI2 are upregulated in patients with IPF and in the murine airway after induction of pulmonary fibrosis by bleomycin treatment [99,100].

Allergic asthma is a chronic inflammatory airway disease triggered by inhalation of environmental allergens, in which inflammatory cells such as mast cells, eosinophils, basophils, innate lymphoid cells, and Th2 cells interact with epithelial cells of the airways, leading to inflammation, mucous overproduction, airway hyperresponsiveness, and tissue remodelling [101]. GWAS and expression studies have linked the Hh pathway to allergic asthma [102–106]. Subjects with asthma exhibited elevated SHH protein in BALF compared to non-asthmatic control subjects [103]. Additionally, Shh signalling may be involved in epithelial–mesenchymal transition and airway remodelling in asthma [107].

Several studies also support the role of Hh signalling pathway in the crosstalk between respiratory and inflammatory cells in the development of allergic asthma [35,108–110]. Recombinant Shh treatment or Smo-inhibitor treatment induced or inhibited respectively epithelial and leukocyte expression of CCL2, an important chemokine for the recruitment and

activation of leukocytes and induction of leukotriene C4 release into the airway, thereby contributing to airway hyperresponsiveness [109]. In vitro and in vivo models showed that reduction of *Ptch1* expression in the lung epithelium decreased cell proliferation and goblet hyperplasia, attenuating mucous production [40]. In mouse models of allergic airways disease, Hh pathway activation in T-cells intensified the severity of Th2 disease by increasing Th2 cell recruitment, cytokine production and influx of eosinophils to the airway [35,108]. Additionally, constitutive deletion of the interferon inducible transmembrane (IFITM) gene family, which are transcriptional targets of Hh signalling in T-cells, led to a reduction in Th2 differentiation in vitro and in severity of murine allergic airways disease in vivo, suggesting that the IFITM proteins may be downstream of Shh signalling in T-cells in driving Th2 inflammation in lung [7,111,112].

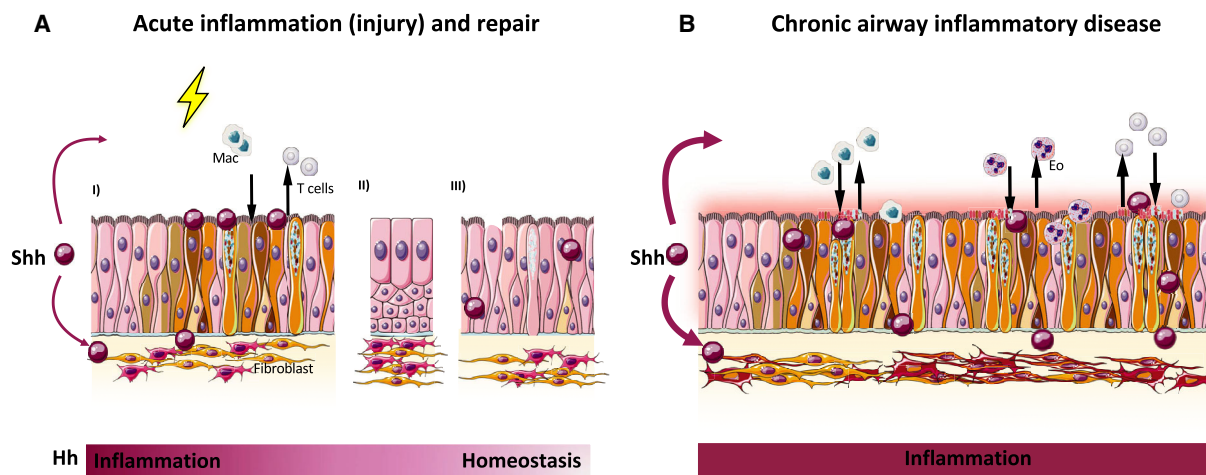
Lung epithelial cells, eosinophils and Clara cells, also respond to Hh signals, possibly contributing to the pathophysiology of the allergic disease [103,108]. Inhibition of the Hh pathway, by Smo-inhibition or treatment with the Shh-neutralizing antibody, has been shown to decrease Th2 responses, cellular infiltration to lung and BAL, and mucous production and also to reduce abnormal remodelling of the airway in mouse models of allergic airways disease [103,109,110].

In summary, Hh up-regulation may be viewed as a natural response to injury to maintain airway homeostasis, but perturbation of normal levels of Shh expression and Hh pathway activation can also contribute to the development and progression of chronic inflammatory lung disease and Th2 inflammation (Fig. 3).

## Conclusions

Shh is essential during the development of many epithelial tissues, including the lung and skin, and after birth its expression is maintained at low levels, where it may be involved in the tissue homeostasis and stem cell/progenitor cell function. Dysregulated or up-regulated Shh signalling is involved in malignant transformation of epithelial tissues, and HH signalling is involved in both skin and lung cancers. However, in response to damage, injury, and disruption of the barrier to the external environment, Shh expression is increased and Shh functions in tissue repair, but also signals to immune and inflammatory cell populations.

Overall, Shh signalling to T-cells appears to dampen adaptive immunity, reducing TCR signal strength, T-cell activation, and Th1 differentiation, but promoting regulatory T-cell and Th2 responses [7,29,33,35,



**Fig. 3.** Hedgehog signalling in Lung inflammation. (A) Upon injury (shown as yellow symbol) (I) Shh is up-regulated in the airway epithelium, signalling to nearby cells and promoting (II) proliferation and tissue repair to re-establish (III) lung homeostasis, upon which Shh levels return to normal. (B) In chronic inflammation, Hh signalling is altered and higher expression of Shh is maintained. This leads to changes in the epithelium (tissue remodelling), and signalling to immune and inflammatory cells, which supports the inflammatory response. Mac, macrophage, Eo, eosinophil.

37,39,53]. This may have important consequences for the immune response to Shh-secreting tumours, as Shh secretion may serve as a mechanism of immune evasion by epithelial tumours, reducing the T-cell response to the tumour and inducing immune regulation.

Additionally, up-regulation of Shh expression in epithelial tissues influences inflammation, and Shh has been shown to have anti-inflammatory action in many different tissues [39,52,91,113–118]. For example, in the skin on induction of mouse atopic dermatitis, Shh signals to increase Treg cells in the skin and to promote their immune regulatory function, protecting against skin inflammation [39]. In contrast, in the lung and airways, Shh has the opposite effect, and its up-regulation drives Th2 inflammation in human and mouse allergic asthma, signalling to induce Th2 differentiation and cytokine production in CD4 T-cells, inflammatory cells of the innate immune system, and airways epithelial cells [35,103,105,107,108,110].

It is unclear why Shh is protective against inflammation and atopic disease in the skin but drives atopic disease in lung and airways, particularly as the same individuals may be susceptible to atopic disease in different tissues at different life stages. Several explanations are possible. First, as Shh can function as a morphogen, which signals for distinct outcomes dependent on strength and duration of signal transduction, it is possible that differences in the levels of Shh upregulation in damaged skin or lung might account for the different outcomes, so that the Shh signal strength to CD4 T-cells in skin would lead to regulatory T-cell

differentiation and immune regulation reducing Th2 inflammation, whereas in the lung the Shh signal strength would lead to Th2 cytokine secretion by epithelium, and Th2 differentiation of CD4 T-cells, driving Th2 inflammation. Second, it is possible that tissue-dependent intrinsic differences in the state of differentiation and activation of immune and inflammatory cells present in the skin and lung on injury account for their different responses to the Shh signal. Third, it is possible that additional unknown environmental signals and queues from the tissue are integrated with Shh signalling to determine whether Shh has anti-inflammatory or pro-Th2-inflammatory action.

The fact that atopic skin disease occurs most often in infancy and early childhood, whereas asthma tends to occur later in childhood, might also relate to developmental differences in the levels of Shh expression at the different sites and to the possibility that failure to produce a robust Shh-dependent regulatory T-cell response in skin in early childhood might increase susceptibility to atopic inflammation at other sites later in life.

The tissue-specific differences between the effect of Shh signalling on inflammation have high-lighted many future areas for research. In addition, it will be important to investigate the potential role that Hedgehog signalling plays in immune and epithelial cell crosstalk during acute and chronic inflammation, during leukocyte migration and during resolution of inflammation and tissue repair.

In summary, recent studies have highlighted the dual role of Shh in barrier tissues after birth, guiding repair

and structural integrity of epithelial structures and at the same time signalling to immune and inflammatory cells to regulate or drive inflammation. Thus, Shh is an important factor in epithelial-immune cell crosstalk in these barrier tissues, and further research into the tissue-dependent mechanisms that underlie its actions will be important.

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## Conflict of interest

The authors declare no conflicts of interest.

## Author contributions

All authors contributed equally to the writing and preparation of this manuscript.

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