

PATHOBIOLOGY IN FOCUS

Take the Wnt out of the inflammatory sails: modulatory effects of Wnt in airway diseases

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Bronchial asthma and chronic obstructive pulmonary disease (COPD) are chronic diseases that are associated with inflammation and structural changes in the airways and lungs. Recent findings have implicated Wnt pathways in critically regulating inflammatory responses, especially in asthma. Furthermore, canonical and noncanonical Wnt pathways are involved in structural changes such as airway remodeling, goblet cell metaplasia, and airway smooth muscle (ASM) proliferation. In COPD, Wnt pathways are not only associated with structural changes in the airways but also involved in the development of emphysema. The present review summarizes the role and function of the canonical and noncanonical Wnt pathway with regard to airway inflammation and structural changes in asthma and COPD. Further identification of the role and function of different Wnt molecules and pathways could help to develop novel therapeutic options for these diseases.

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Obstructive airway diseases are the most prevalent chronic lung diseases. Two entities, bronchial asthma and chronic obstructive pulmonary disease (COPD), are described under this term. Asthma is defined as the presence of airway obstruction, increased reactivity to inhaled bronchoconstrictors (airway hyperreactivity), and airway inflammation (<http://www.ginaasthma.org>). This syndrome can be found in many different clinical presentations, and various clinically or immunologically categorized phenotypes have been described in the past years.¹ COPD is characterized by persistent airflow limitation, which is usually progressive over time (<http://www.goldcopd.com>). In addition, inflammatory and structural changes can be found in the airways and lung. This can be apparent as small airway disease (obstructive bronchiolitis) or destruction of lung parenchyma (emphysema). Chronic airway inflammation has been described in asthma as well as in COPD. However, the underlying cellular mechanisms are distinctively differing between asthma and COPD.

Several different asthma phenotypes have been described. It has been speculated that these asthma phenotypes also differ in their pathobiology.² By far the best understood immunological pathways are in allergic asthma. Indeed, in allergen-induced airway inflammation, T-helper type 2 (Th2) cells have a pivotal role. This type of asthma often develops in

childhood and is associated with atopic constitution and with other manifestations of atopic diseases such as allergic rhinitis, atopic dermatitis, and food allergy. Th2 cell polarization and also activation is dependent on dendritic cells (DCs), which present the specific allergens. Indeed, following allergen exposure in the lung, the protein is taken up by DCs, which migrate to the draining lymph node, where antigen-specific T cells are activated. These cells then migrate to the lung and induce an inflammatory reaction by the secretion of proinflammatory mediators such as the Th2 cytokines (interleukin-4 (IL-4), IL-5, and IL-13). This inflammatory response is characterized by the presence of eosinophils. A certain transcription factor, the *trans*-acting T-cell-specific transcription factor GATA-3, has been found to be central for the function of Th2 cells,³ and recent findings have strengthened the role of GATA-3 as a master switch for allergen-induced asthma. Indeed, direct blockade of GATA-3 resulted in decreased early and late responses in patients with allergic asthma following allergen challenge.⁴ In recent years, there has also been increasing attention to the role of the airway epithelium in mediating the inflammatory responses in the airways. It is now well recognized that epithelial-derived mediators such as TLSP and IL-33 are critically involved in the development and chronicity of allergic airway disease.^{5–7}

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In COPD airway, inflammation is mainly induced by inhaled cigarette smoke or other noxious particles. The pathological changes are found in the airways, lung parenchyma, and pulmonary vasculature. Increased numbers of different cells such as neutrophils, macrophages, and CD8⁺ T cells and a wide variety of inflammatory mediators have been reported.⁸ In addition, further characteristics of the disease are manifold structural changes in the lung, ranging from fibrosis around the small airways to loss of lung parenchyma, which results in a rarefaction and instability of the small airways.⁹ There is increasing evidence that in COPD the airway epithelium has an important role in orchestration of inflammatory and immune responses. In addition, epithelial function seems to have a key function in tissue remodeling.¹⁰

Unraveling the underlying immunological processes is important, not only to understand the disease but also for the development of novel therapeutic approaches. Indeed, for asthma novel antibodies have been developed to target Th2-induced inflammation and several of these antibodies are close to be approved for clinical treatment.¹¹ Therefore, research to further elucidate the underlying mechanisms of airway diseases is necessary. Discovered over 30 years ago, Wnt-dependent effects were mainly described during embryogenesis of model organisms such as sea urchin, *Drosophila*, and *Xenopus*. Using recombinant ligands, specific inhibitors, and also transgenic mouse models, it is now clear that Wnt pathways are not only involved in developmental processes but also have an essential role in repair processes and regulation of immune responses. The present review summarizes the current knowledge about the role and function of Wnt pathways on immunological and structural changes observed in asthma and COPD.

WNT PATHWAYS

Wnt proteins are a diverse family of secreted lipid-modified cysteine-rich proteins and this structure has made a detailed characterization for a long time almost impossible. In 2003, Wnt3a was purified and the hydrophobic structure common for Wnt proteins was identified.^{12,13} Functionally, because of their biochemical properties, Wnt ligands are short-range signaling molecules.¹⁴ So far, 19 different Wnt ligands have been identified and structurally described.¹⁵ Wnt ligands are recognized by Frizzled (Fzd) receptors, a family of seven-pass transmembrane receptors,¹⁶ which are commonly associated with coreceptors such as the LDL receptor-related proteins 5 and 6,¹⁷ and also other coreceptors such as ROR or Ryk¹⁴ are described. So far, 10 human Fzd genes have been discovered. Ligation of the trimeric Wnt/Fzd/coreceptor complex results in cell signaling. Depending on ligand, receptor, and coreceptor composition, different downstream pathways can be activated. These pathways can be divided into a canonical pathway, now referred to as Wnt/ β -catenin, and two noncanonical pathways, which are independent of β -catenin (the planar cell polarity (PCP) and the Wnt-Ca²⁺ pathway).¹⁴

However, cellular context, receptor equipment, and present Wnt ligands can change the normally preferred pathway and induce variations leading to a different unexpected outcome.¹⁵

In the current understanding, Wnt1 and Wnt3a ligands act as activators of the Wnt/ β -catenin pathway, whereas Wnt5a and Wnt11 are perceived as examples for noncanonical pathway effects.¹⁸ The Wnt/ β -catenin pathway has been well characterized by its strong dependency on β -catenin. Under steady-state conditions, cytoplasmatic β -catenin is degraded by the proteasome, a process that is strongly controlled by a glycogen synthase kinase-3 β (GSK-3 β) involving destruction complex. Upon Wnt ligand receptor/coreceptor (LRP5/6) binding, this complex becomes inactivated, leading to cytoplasmic accumulation and subsequent nuclear translocation of β -catenin. In the nucleus, it forms together with T-cell factor and lymphoid enhancer-binding factor, a transcription complex, able to regulate the expression of target genes.¹⁸

The noncanonical pathways are completely independent of β -catenin-mediated gene expression. The nomenclature is so far not consistent. Indeed, different defined pathways, such as PCP and Wnt5A-ROR, demonstrate comparable signal cascade and could therefore be regarded as virtually identical.^{19–21} The PCP pathway is involved in developmental processes such as regulation of cell polarity, movement, and orientation. This pathway signals via small GTPases such as RHOA and RAC1 and the JUN-N-terminal kinase leading to the activation of JUN-N-terminal kinase depending transcription factors such as ATF2.²² Interestingly, the Wnt/ β -catenin and PCP pathways seem to have opposed regulation so that activation of one pathway will suppress the other.²³ Wnt5a and Wnt11 are also regarded as prototype ligands for the Wnt-Ca²⁺ pathway. In the presence of different Fzd receptors, they are able to induce a calcium-dependent signaling cascade via phospholipase C and inositol trisphosphate activation.^{24–27}

CELL POPULATIONS INVOLVED IN AIRWAY INFLAMMATION

In allergic asthma, several cell types are involved in the inflammatory reaction towards harmless antigens. In healthy subjects, inhaled exposure to an antigen induces tolerance and prevents the development of sensitization towards this harmless molecule. In contrast, under certain circumstances such as the presence of certain danger signals, an orchestrated immune response develops including cells forming the innate (eg, macrophages, DCs, mast cells, innate lymphocytes) and the adaptive immune systems (T cells), and also of structural cells (eg, epithelial cells), which results in the development of specific T- and B-cell responses against the allergen.^{28–31} In these sensitized individuals, exposure with the allergen results in specific immunological responses in the lung, in which mast cells and DCs modulate the adaptive immune responses, which is characterized by a Th2 response, including IL-4, IL-5, and IL-13, and accumulation of eosinophilic

granulocytes in the airway wall and lumen.^{32–35} If these immune responses persist in the airway wall, structural changes, such as goblet cell metaplasia, basement membrane thickening, hypertrophy of ASM, and subepithelial fibrosis, can occur and these structural changes are summarized under the term airway remodeling.²

Dendritic Cells

DCs are commonly regarded as ‘the’ professional antigen-presenting cell and are also in the lung essential to induce proper adaptive immune responses against pathogens or tolerance against harmless antigens. Depending on the degree of activation, the intracellular status and the secreted mediator DCs are the important checkpoint of either activating or suppressing the immune response. Human as well as murine DCs express a variety of Wnt receptors and are so susceptible for canonical as well as noncanonical Wnt signaling.³⁶ Exposition to Wnt ligands and activation of the canonical pathway has been linked to DC-mediated induction of tolerance.³⁷ Interestingly, exposition to Wnt does not lead to a change in the expression of activation markers on DCs such as CD80, CD86, CCR7, or MHCII.³⁷ Still, *in vitro* exposure of isolated DCs with either Wnt3a (Wnt/ β -catenin ligand) or Wnt5a (noncanonical ligand) resulted in the production of different anti-inflammatory mediators, with Wnt3a inducing TGF- β expression, whereas Wnt5a induce the additional expression of IL-10.³⁸ Furthermore, coculture of Wnt-treated DC with naïve T cells resulted in the induction of FoxP3-expressing regulatory T cells³⁸ and also exposure to Wnt1 markedly reduced the activation of effector T cells by DCs in coculture experiments.³⁹ Similar results were observed in a human cell coculture system. Exposure to Wnt5a resulted in increased secretion of IL-10 in blood-derived monocytes.⁴⁰ DCs exposed to this ligand express more indolamin-2, 3-dioxygenase, a tryptophan-degrading enzyme with immune regulatory properties, and induce more regulatory T cells.⁴¹

Taken together, these data strongly suggest that Wnt/ β -catenin as well as noncanonical Wnt ligands have the ability to shape DCs to a tolerogenic status and thus preventing or regulating adaptive T-cell responses. This is supported by *in vivo* studies; a murine model of chronic inflammatory bowel disease showed that the expression of β -catenin in DCs is important for the production of anti-inflammatory mediators such as retinoic acid-metabolizing enzymes, IL-10, and transforming growth factor- β .⁴² In addition, T cells isolated from small and large intestine of animals, which lacked the expression of β -catenin in DCs, demonstrated an increased inflammatory phenotype, and isolated DCs showed a reduced ability to induce a Foxp3-expressing regulatory T cells but an enhanced capacity to induce IL-17 and IFN- γ -expressing effector cells. These data demonstrated for the first time that Wnt signaling, and especially the Wnt/ β -catenin pathway, has an important role in the induction/maintenance of a tolerogenic DC phenotype *in vivo*. Interestingly, the Wnt/ β -catenin signaling pathway seems to be also important for

other autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis.⁴³ Indeed, mice that lacked the expression of the Wnt coreceptors LRP-5/6 on DCs demonstrated an increased EAE phenotype. Moreover, cells from those animals fail to induce IL-10 production and demonstrate increased secretion of IL-17 and IFN- γ . Furthermore, mice that express active β -catenin or are treated with a β -catenin agonist were more protected from developing EAE associated with reduced IL-17 and IFN- γ production and increased IL-10 expression. Indeed, these results strongly suggest that Wnt/ β -catenin-dependent signaling has an immune-suppressive function *in vivo*. However, if these findings in the gut or the brain could be extrapolated to different organ systems such as the lung was unclear.

T Cells

T cells are the central effector cells of the adaptive immune system. Controlling humoral and cellular responses, they represent long-lasting effective defense mechanisms against viral and bacterial infections. However, T cells also have an important role in autoimmunity and hypersensitivity responses. Indeed, allergen-specific CD4⁺ T-helper cells are responsible for induction, maintenance, and chronicity of diseases such as allergic asthma. Moreover, induction of different subsets of T cells expressing specific cytokine profiles is discussed as the main reason for the development of heterogeneous asthma phenotypes.² Induced by DCs, effector T-cell responses are mainly controlled by regulatory T cells (Tregs). These cells are important for suppression of adaptive immune responses and thus for the clearance of immune responses by acting via direct cell contact-dependent or indirect cytokine-dependent mechanisms.^{44,45} Moreover, because of the modulation of DC responses, they can also control the development of an adaptive immune response. So far, there are several *in vitro* studies demonstrating an immune-suppressive effect of Wnt signaling, which is mediated by a direct suppression of effector cells and stabilization of Tregs. Indeed, stabilization of β -catenin in murine Treg supports their survival and their suppressive capacity.⁴⁶ In addition, stable β -catenin expression in effector T cells induces anergy and prevents inflammatory responses.⁴⁶ Treatment of polyclonal stimulated human T cells with either Wnt3a or a GSK-3 β inhibitor prevented polarization of these cells.⁴⁷ Moreover, blocking of GSK-3 β reduces proliferation and cytokine secretion. These effects could also be observed in cytotoxic CD8⁺ effector cells.⁴⁸ Still, Wnt signaling seems to be involved in T-cell homeostasis, as prevention of β -catenin degradation results in lymphopenia, which is mediated by spontaneous activation of naïve T cells in the periphery.⁴⁹ Taken together, these data demonstrate direct interactions of Wnt ligands with T cells. Especially, T-cell survival and polarization are affected by Wnt ligands and most of the studies suggest that exposure to Wnt ligands induces a suppressive T-cell phenotype.

Macrophages

Macrophages represent one of the first defensive lines of the immune system and are important for the appropriate induction of innate immune responses. Owing to their variety on proinflammatory mediators, they have an important role in the defense against invading pathogenic organisms. However, to prevent harmful effects from an excessive immune response, a well-orchestrated interplay of inflammatory and regulatory mechanisms is essential. Similar to DCs, Wnt signaling has heterogeneous functions in macrophages, which seem to be dependent on the phase of an inflammatory response. Indeed, under homeostatic conditions Wnt5a is necessary to prolong survival of macrophages and keeping the network for immediate antimicrobial immune responses intact.⁵⁰ In contrast, during acute inflammation Wnt5a is also involved in inducing anti-inflammatory effects from macrophages to prevent an excessive and harmful immune response.^{50–53} These data imply that Wnt signaling is important to avoid overwhelming harmful inflammatory reactions in responses to acute infections. In contrast, Wnt ligands have shown no effect on the differentiation into alternative activated M2 macrophages. M2 macrophages themselves have been described to produce Wnt ligands and by this pathway could support the development of a tolerogenic milieu.⁵⁴ Therefore, comparable to findings in Wnt signaling in DCs, activation of the Wnt pathways in macrophages lead to suppressive responses, especially during inflammatory reactions, thereby preventing excessive inflammation and potentially supporting resolution of inflammation. Again, the immunosuppressive properties of Wnt ligands seem to be strongly context-dependent, as it has been demonstrated that Wnt3a enhances the secretion of TNF- α in an LPS-activated macrophage cell line.⁵⁵

IMMUNOLOGICAL ROLE OF WNT PATHWAYS IN ASTHMA

In contrast to the well-identified function of different Wnt pathways in embryogenesis and structural development, there is scarce data on the role and function of Wnt expression in airway diseases. Several *in vitro* studies have demonstrated that Wnt ligands can modulate functions of DCs, T cells, and macrophages, suggesting that Wnt ligands could have a profound impact on innate and adaptive immune responses. As discussed above, pro- and anti-inflammatory properties of Wnt ligands were observed depending on cell type, Wnt ligand, and experimental setup. As mentioned above, evidence from models of inflammation in the brain or gut has already suggested that Wnt ligands display immunosuppressive functions *in vivo*. However, if these findings could be extrapolated to different organ systems such as the lung was unclear. Interestingly, in human gene expression studies, expression of several Wnt-associated molecules, such as Wnt1-inducible factor signaling pathway protein-1 and Wnt inhibitory factor-1, were associated with impaired lung function.⁵⁶ In addition, expression of Wnt3a, Wnt5a, Wnt6, Wnt10, and Fzd5 were correlated with a Th2 signature in

human asthmatic airways.⁵⁷ However, these studies describe an association without elucidating the pro- or anti-inflammatory function of these ligands. Recent studies investigated the role and function of Wnt in the context of allergic airway inflammation using an inducible lung-specific over expression of Wnt1. Indeed, induction of Wnt1 before and during airway challenges reduced airway inflammation and airway hyperreactivity, both hallmarks of allergic asthma.³⁹ These effects were detectable in prophylactic situation (increased Wnt expression before the first development of allergic airway disease) and also in a therapeutic context (increased Wnt expression in developed airway disease). Further analysis of the immunological responses showed no change in the proportion of regulatory T cells in lymph nodes or in lung tissue. Instead, the suppressive effects of Wnt1 were related to suppressed migration of antigen-loaded DCs from the lung to the draining lymph nodes and their activation state. Indeed, application of *in vitro* generated allergen-exposed DCs from wild-type animals was effective in overcoming Wnt1-induced suppression of allergic airway disease. Overall, these results suggest that Wnt1 prevents the activation of pulmonary DCs and by this pathway suppress the development and aggravation of allergic airway disease.

It has been well described that the chemical element lithium is strongly associated with the canonical Wnt pathway. Similar to the effect of canonical Wnt ligands also lithium chloride (LiCl) effectively suppresses the β -catenin destruction complex by inhibiting one of its central components GSK-3 β .⁵⁸ Therefore, LiCl has been widely used as a surrogate compound to assess the involvement of the Wnt/ β -catenin pathway.^{59–64} Interestingly, the current knowledge of the Wnt pathway can now explain clinical observation made even before the Wnt pathways have been described. Early case reports already demonstrated that treatment of patients suffering from chronic schizophrenia and asthma with LiCl not only reduced the manic episodes but also caused a reduction of asthma attacks and the use of asthma-specific medication,⁶⁵ and other clinical observations showed that stopping a long-lasting LiCl therapy in a patient-induced increased airway hyper-responsiveness and asthma symptoms.⁶⁶ The important role of GSK-3 β -mediated signaling on the development of asthma was also confirmed in a murine model of allergic airway disease. By blocking GSK-3 β before antigen challenge by application of a specific GSK-3 β inhibitor, airway inflammation and other features of the asthma phenotype were significantly reduced.⁶⁷ In addition, recent studies using LiCl in a murine model of allergic airway disease showed that treatment of sensitized animals before and during allergen challenge with LiCl reduced airway inflammation, hyperresponsiveness, and mucus production.³⁹ These findings add a mechanistic explanation to the observed effects in humans.

In summary, in allergic asthma the GSK-3 β - and β -catenin-dependent canonical Wnt pathway has an immune modulatory function. The immune-suppressive properties of Wnt/ β -catenin

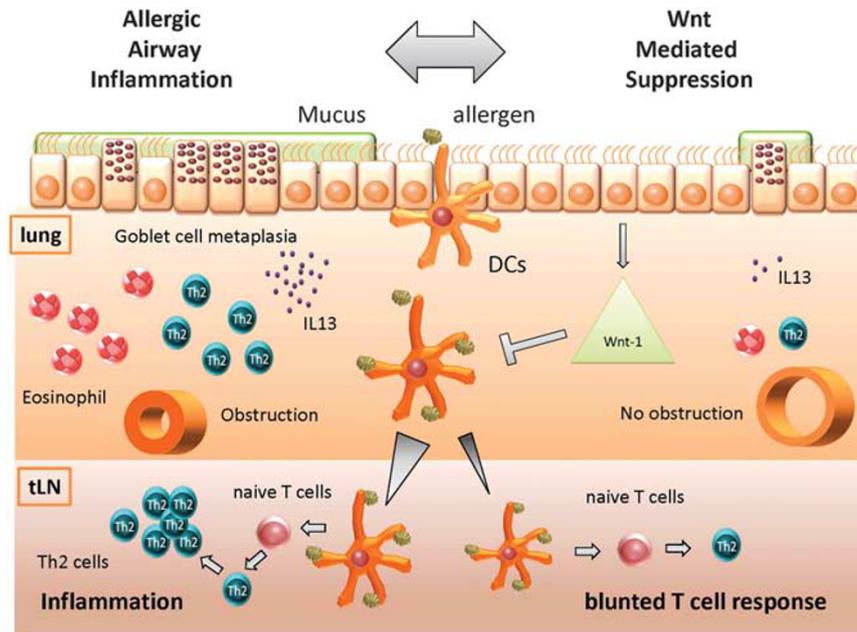


Figure 1 Wnt-mediated regulation of allergic airway diseases. Allergic airway disease is driven by misguided immune responses towards a harmless aeroallergen. Besides, dendritic cells (DCs) are central for the induction and exacerbation of an allergic airway disease. DCs take up allergens in the lung, process them, and migrate to the draining lymph node. Here depending on their activation state, they induce anergy or activation of antigen-specific T cells. Once induced, activated effector T cells migrate to the lung and secrete proinflammatory cytokines such as interleukin-13 (IL-13), which lead to the generation of an acute inflammation and structural changes. Wnt1 modulates the migration and phenotype of allergen-loaded DCs. Wnt1-exposed DCs fail to induce an effector T-cell response and therefore also the further features of the disease such as airway inflammation and goblet cell metaplasia.

ligands include the modulation of DC activation, migration, and antigen presentation, and by this pathway prevent induction and secretion of proinflammatory Th2 cytokines and eosinophilic airway inflammation (Figure 1).

Role of WNT Pathways in Airway Remodeling

In asthma and COPD not only inflammatory but also structural changes can be found in the airways. Repetitive or persistent exposure of the airway epithelium by allergen contact, air pollutants, cigarette smoke, or cycles of infection leads to chronic inflammation. This epithelial damage can be directly induced by proteolytic activity of allergens,^{68–70} histamine,⁷¹ or pollen.⁷² Exposure and stress of the epithelium leads to the production of different cytokines, which induce certain immune responses, and can also be involved in aberrant or uncontrolled tissue repair.^{73,74} Remodeling processes in the airways include hypertrophy and migration of ASM, goblet cell metaplasia, basal membrane thickening, subepithelial fibrosis, and airway neovascularization.

Mucus production is one of the features of allergic airway disease. MUC5AC and MUC5B are the principal gel-forming mucins in the lung and are secreted by goblet cells and submucosal glands.⁷⁵ The molecular mechanisms leading to goblet cell differentiation contain several signaling pathways

such as EGFR signaling, STAT6 activation via IL-4 and IL-13, and the Notch signaling pathway. Both EGFR and IL-4 receptor activation lead to the expression of MUC5AC, whereas the activation of Notch signaling is able to inhibit or to induce MUC5AC expression.⁷⁶ The current hypotheses is a *trans*-differentiation of existing ciliated and Clara cells to goblet cells, with changes of their form to a more slim and elongated shape. Moreover, the increased number of goblet cells leads to a rise of viscous mucus production, which exacerbates the diminished respiratory airflow of the patients.⁷⁷ *In vivo* experiments in mice were able to detect a connection between Wnt/ β -catenin signaling and diminished goblet cell metaplasia. Pharmacological inhibition of GSK-3 β significantly reduces expression of MUC5AC in a murine model of allergic airway disease.⁶⁷ In addition, a dose-dependent reduction of IL-13 with rising doses of the GSK-3 β inhibitor was detected. In addition, other studies have shown an inhibitory effect of Wnt on goblet cell metaplasia. Both overexpression of Wnt1 in the airways and treatment with LiCl resulted in diminished goblet cell metaplasia.³⁹

Particularly, the increase of smooth muscle mass induced by cellular hyperplasia and hypertrophy is responsible for chronic airflow limitation and increased bronchial hyperresponsiveness. Interestingly, Wnt/ β -catenin signaling is involved in proliferation of ASM *in vivo* and *in vitro*.

Stimulation of ASM with growth factors such as PDGF increases the nuclear β -catenin concentration and is associated with enhanced ASM proliferation, and this effect is dependent on β -catenin.⁷⁸ Furthermore, β -catenin expression is increased in proliferating human ASM cells.⁷⁹ Besides these *in vitro* observations, there are a few *in vivo* models, which confirm the β -catenin effect on ASM proliferation. On the one hand, Wnt7b/ β -catenin signaling is an important factor for ASM development,⁸⁰ and on the other hand, β -catenin induces proliferation of ASM.^{81,82} Moreover, not only proliferation but also contraction of ASM is affected by Wnt/ β -catenin signaling. In ASM, β -catenin exists as membrane-associated protein in a complex with actin filament facilitating cell–cell contacts. The activation of Wnt/ β -catenin signaling enhances the expression of *de novo* β -catenin and augmented methacholine-induced contraction.⁸³ These results let us assume that inhibition of Wnt/ β -catenin signaling could prevent ASM proliferation and improve AHR.

Many of the insights of the function of Wnt in the context of repair processes in the lung have been identified in models of acute epithelial and lung injury,^{84–87} and these studies show an important role for the canonical β -catenin-dependent Wnt pathway during repair processes in the lung. Another mechanism involved in epithelial repair is the so-called epithelial–mesenchymal transition (EMT). EMT is well described in different processes such as organ development, cancer, fibrosis, and tissue repair.^{88,89} During EMT epithelial cells differentiate into mesenchymal–fibroblast-like cells. This transition is characterized by reduction of their cell-to-cell contacts, the downregulation of certain markers (such as E-cadherin, claudin, or mucin-1), enhanced cell migration, and induction of mesenchymal gene expression (such as Snail 1, fibronectin, α -smooth muscle actin (α -SMA), vimentin, and cyclin D1).^{90,91} Different reports suggest that EMT is linked to airway remodeling in asthma.^{70,92} Interestingly, EMT is regulated by the Wnt/ β -catenin and the noncanonical Wnt signaling pathway.^{93–95} Also in the lung, Wnt signaling is involved in the regulation of EMT. Especially the cross-talk between Wnt/ β -catenin signaling and TGF β , which is a master regulator of EMT, seems to be pivotal in mediating EMT.^{96,97} Indeed, a stimulation of cultured cells with an allergen induces β -catenin, especially in the presence of TGF- β . This synergistically leads to a higher expression of the mesenchymal markers fibronectin and vimentin.⁷⁰ These observations suggest that Wnt/ β -catenin and TGF- β are important mediators for the induction of structural changes triggered by EMT in the airways of asthma patients following repeated allergen exposure. Given the so far limited amount of data, further confirmation of these findings would strengthen this concept. Still, the functions of Wnt pathways in mediating EMT are supported by the finding that pharmacological activation of β -catenin in human bronchial cells or overexpression in basal cells upregulates the expression of EMT markers in these cells.⁸⁵

Function of Wnt Pathways in COPD

In addition, structural changes in the lung of COPD patients seem to be associated with Wnt signaling. One feature of COPD is the development of emphysema with progressive impairment of respiratory functions. Besides, changes in the airways can also be detected, such as mucus hypersecretion by hyperplastic goblet cells and the increased thickening of the wall of small airways.⁸ Furthermore, EMT is a process involved in remodeling of airways in COPD. Again, Wnt signaling is critically involved in this process. Indeed, Wnt3a and activation of canonical Wnt/ β -catenin pathway is upregulated in epithelial cells following nicotine exposure.^{98,99} The activation of β -catenin was accompanied by an increase of mesenchymal markers such as α -SMA, vimentin, type 1 collagen, and MMP-9, as well as a downregulation of E-cadherin. The knockdown of Wnt3a prevents the observed effects.¹⁰⁰

Another feature of COPD is loss of peripheral lung tissue and the development of emphysema induced by cigarette smoke and environmental factors in people with genetic predisposition.^{101–103} Emphysema is characterized by alveolar airspace enlargement, parenchymal destruction, and impaired pulmonary repair.^{8,104} Currently, there are no therapeutic agents available, which induces lung regeneration. A potential option for the future could be the reactivation of signaling pathways that are active during lung development to induce epithelial repair in the damaged areas similar to the Wnt/ β -catenin pathway.^{70,105,106} In human lung samples of patients with COPD, a reduction of nuclear β -catenin is detectable.⁶⁴ In addition, decreased Wnt/ β -catenin activation has been described in different murine models of emphysema and treatment with LiCl attenuated airspace enlargement and prevents the development of emphysema.⁶⁴ This protective role of Wnt for the development of emphysema is supported by the finding that Frizzled-related protein-1, an inhibitor of Wnt signaling, induces proteases, which are associated with emphysema formation and tissue destruction.¹⁰⁷ If modulating Wnt signaling might pose an interesting therapeutic approach in COPD is under investigation. Very recent experimental findings are promising. In cultures of COPD patient-derived lung tissue, increased Wnt/ β -catenin signaling attenuated several pathological features of the cells, suggesting a potential therapeutic effect of increased Wnt signaling.⁶²

CONCLUSION

In summary, recent studies suggest important immune modulatory effects of Wnt molecules on inflammatory as well as structural processes in the airways in asthma and COPD (Figure 2). With regard to inflammatory processes, Wnt pathways mainly seem to elicit regulatory and suppressive functions. Indeed, Wnt/ β -catenin and also noncanonical Wnt ligands directly affect DCs, T cells, and macrophages. The exposure to Wnt ligands seems to suppress the development of allergic airway disease and these effects mainly seem to be mediated by effects on DCs. In addition,

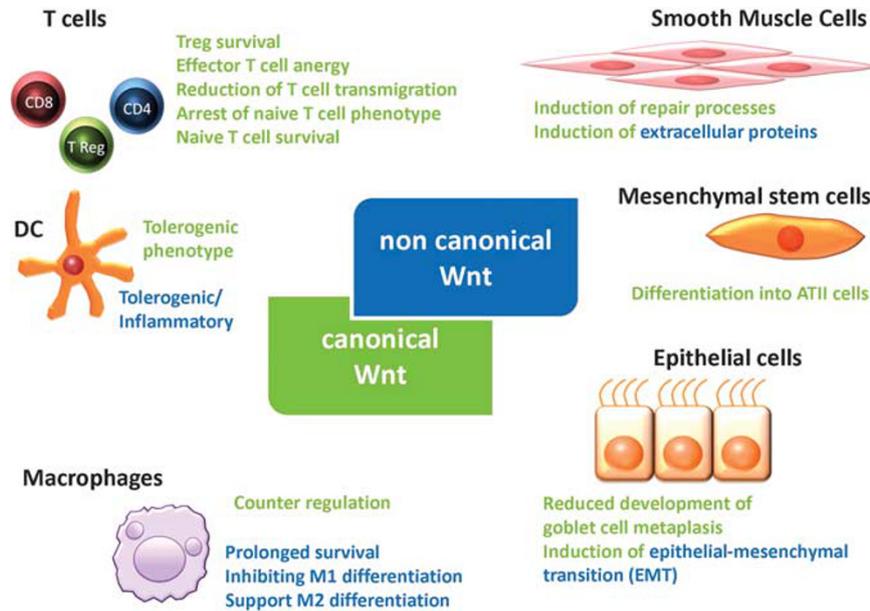


Figure 2 Impact of Wnt ligands on immune and structural cells. Effects of canonical ligands on different immune and structural cells are displayed in green, whereas effects of noncanonical Wnt ligands are displayed in blue. If similar effects are induced by both canonical and noncanonical ligands effects are displayed as green and blue.

Wnt ligands are also involved in structural changes in the airway and have been implicated with mucus production and EMT. Interestingly, mucus production seems to be inhibited by Wnt ligands, whereas Wnt ligands seem to be a driver of EMT. Further research is needed to dissect the precise role of the different Wnt ligands in this context. Analyzing the expression of different Wnt ligands, their regulators and changes in the receptor expression in chronic allergen challenge models could help to further understand the dynamics of different Wnt molecules in this setting. In addition, usage of gene-deficient animals as well as long-term overexpression of Wnt ligands in the airways could help to further define the long time effects of these ligands in the remodeling process. The increasing possibilities of targeting these genes in an organ-specific manner using, for example, Cre-recombinase techniques will further help to unravel the pathophysiological function of the different Wnt pathway components. Our increasing knowledge about the effects of Wnt ligands, especially in allergic airway disease or in COPD, could make these pathways an interesting target for pharmacological interventions. Despite these promising results, a careful evaluation is mandatory, given the role and function of Wnt pathways in tumorigenesis.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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