WNT and β-catenin signalling in airway smooth muscle: emerging concepts for asthma

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Chapter eight

General discussion
Preface

Airway remodelling is defined as the morphological change in structure or composition of the airway wall. This may include any or all parts of the wall. Morphometric studies of healthy and diseased lungs have made it abundantly clear that the airway wall thickens in asthmatics, most notably in severe and fatal asthma. This is particularly true for the airway smooth muscle (ASM) bundle, although other parts of the airways also remodel, including mucous gland hypertrophy, bronchial microvascular remodelling, subepithelial fibrosis and epithelial changes among which cell detachment and goblet cell hyperplasia. Until recently, airway remodelling was considered to be a secondary phenomenon that develops later in life, resulting only because of persistent and ongoing inflammation that characterizes the asthmatic lung. This idea has been challenged due to a number of critical observations. To name a few, infant children who display airflow limitation at birth are more likely to develop asthma later in life. Moreover, an increase in ASM and basement membrane thickness is observed in children, even before asthma is diagnosed. These and other observations, as we have laid out more comprehensively in chapter 1 and chapter 2, have put forward the notion that airway remodelling in asthma can develop in parallel to inflammation, and may in some patients even be a determinant rather than a consequence of inflammation. It is worth mentioning here that not every aspect of airway remodelling in adult asthma is represented in the same way in children. For example, while epithelial shedding has been demonstrated in both adults and children with asthma, asthmatic epithelial cells actively proliferate in adults but not in children. Furthermore, epithelial shedding is also observed in atopic children without asthma and may thus reflect a symptom of allergy instead. In contrast, preschool children who displayed severe wheeze and had increased ASM mass have a significantly higher risk to develop asthma at school age. Only ASM volume could predict the development of asthma, whereas reticular basement membrane thickness, subepithelial eosinophil and ASM mast cell numbers could not. These findings emphasize the importance of the ASM, not only as a predictor of subsequent asthma development, but possibly also as a causal factor of the disease.

In this thesis we built on the premise that WNT signalling is a possible
candidate that underlies the altered ASM state in asthma. The β-catenin independent signalling mediator WNT-5A has recently been identified as a key player in airway remodelling. It is highly expressed in human ASM cells, and even more so in asthmatic ASM. ASM also actively utilizes β-catenin as a transcriptional activator, and there is a substantial amount of cross-regulation between WNT components and the transforming growth factor-β (TGF-β) signalling axis (another important pathway that is integral to airway remodelling). Although it is clear from this work that WNTs and β-catenin are involved in ASM remodelling, many questions remain unanswered. In this thesis we expanded on the roles of WNT and β-catenin signalling within the context of smooth muscle pathophysiology and asthma. In the following sections we will briefly go through our findings and discuss their relevance and implications within the field.

**External modulators of airway smooth muscle contractility**

Airway hyperresponsiveness (AHR) is a hallmark feature of asthma, which is characterized by an exaggerated response of the ASM to contractile stimuli. The contractile state of airway smooth muscle in asthma is directly modulated by a variety of extracellular agonists acting on specific receptors located in the plasma membrane of ASM cells. For example, parasympathetic activity is increased in airway inflammation, and pre-junctional M2 receptors (involved in negative feedback control of acetylcholine (ACh) release, the main neurotransmitter of the parasympathetic nervous system) have been observed to be dysfunctional in some asthmatics. In addition to ACh, several cell types located in the airway wall, such as mast cells, inflammatory cells, epithelial cells, and airway myocytes themselves, can release mediators that induce ASM contraction. Mast cells in asthmatics can be activated to secrete contractile mediators, such as histamine and cysteinyI leukotrienes, of which the latter is also abundantly expressed by eosinophils, along with prostaglandin D2. Epithelial cells can secrete endothelin-1 and prostaglandin E2, that can modulate airway contraction. Stimulation of their cognate receptors activates a cascade of intracellular events that culminate in an increase of cytosolic Ca²⁺ by the release from internal stores, as well as influx from the extracellular compartment. This increase in cytosolic Ca²⁺ activates the contractile apparatus.
Chapter nine

The relevance of intracellular Ca\(^{2+}\) release in the airway smooth muscle within the context of airway disease like asthma, has been discussed in detail in chapter 3.

**Intrinsic smooth muscle changes**

Apart from extracellular mediators that can induce ASM contraction in asthma, a large bundle of work has focussed on intrinsic changes within the ASM itself. However, insights into the mechanisms that may underpin altered contractility of ASM are not fully conclusive. Some studies, but not all, have shown increased expression of myosin light chain kinase (MLCK) in ASM samples from sensitized individuals and asthmatics. Similarly, there may be increased abundance of myosin heavy chain in asthmatic ASM, however not all studies prove this to be the case. This has shifted interest in the field towards factors that are not a direct constituent of the contractile apparatus, but can influence contraction nonetheless. A recent paper has added credibility to this view, where laser dissected airway smooth muscle cells from asthmatics and healthy or atopic individuals were sequenced and screened for differential gene profiles. Among the results were genes that were not part of the contractile machinery, but correlated strongly with AHR nonetheless. A proposed mechanism that may underlie these seemingly incongruous results are events that influence actin dynamics, which as a result can influence ASM contraction. \(\beta\)-catenin independent WNT signalling has been shown to affect cytoskeletal organization, and WNT-5A expression is increased in asthmatic ASM. We hypothesized WNT-5A could modulate ASM contraction via autocrine signalling, and in chapter 4 we addressed this topic in more detail. We later expanded on these results in chapter 5, using WNT-11, another \(\beta\)-catenin independent WNT ligand. We demonstrated that WNT-5A can increase maximum isometric tension in bovine tracheal smooth muscle strips, and showed that WNT-5A is preferentially expressed in contractile human airway myocytes compared to proliferative cells, suggesting an active role in maintaining contractility. Furthermore, both WNT-5A and WNT-11 treatment resulted in increased actin polymerisation through activation of Rho kinase (ROCK). These findings may be relevant for AHR in asthma, as they provide a possible explanation for how ASM cells in asthma can maintain contractility without increasing components of the
contractile machinery. These results are consistent with the findings that asthmatic ASM responds differently to strain induced by tidal breathing, which in healthy individuals results in a bronchodilatory effect that can alleviate airway narrowing. This response is largely absent in asthmatics, and elegant studies by Mitchell and Solway have indicated that actin polymerization might underlie this response. In healthy individuals, persistent bronchodilation induced by deep inhalation reflects a plastic deformation of contracted airway smooth muscle cells. In light of this, contracted airway smooth muscle behaves more or less like chewing gum. When stretched it remains lengthened, and will only shorten slowly and partially after the initial stretch. In contrast, asthmatic ASM, which contains long and thick actin filaments that act in parallel to myosin filaments, are able to maintain intercalating myosin filament arrangements upon stretch, and thus allow most of their capacity to generate force. As an analogy, in this way asthmatic ASM behaves much more like elastic rubber, which lengthens while a stretching force is applied, but quickly assumes its original length upon force release. A more detailed overview of this model is portrayed in figure 1. If this model holds true, then targeting β-catenin independent WNT signalling may have profound effects on AHR in asthma, as this would result in relengthening of contracted airway smooth muscle and subsequent reversal of airway narrowing. Given the ubiquitous importance of actin filaments in a wide range of cell types, it might be challenging to design intervention strategies that inhibit actin turnover selectively within the ASM, without disrupting other important actin functions in the lung, like maintenance of airway epithelial or vascular endothelial barrier integrity. However, WNT ligands are diversely expressed by different cell types, and based on our findings that both WNT-5A and WNT-11 can modulate actin dynamics, a preferred WNT target may be one that is selectively expressed in ASM cells, but not in others. Future studies will have to address this point.
Figure 1. Proposed model of contractile filament rearrangement during stretching of contracted airway smooth muscle cells, with short actin filaments prior to stretching (A) or long actin filaments prior to stretching (B). Actin filaments are shown as orange lines, dense bodies are shown as red ovals, barbed-end actin-capping proteins are shown as green crescents, and myosin filaments are shown in blue. (A) Continued contraction after stretching of smooth muscle cells with short actin filaments, as seen in healthy individuals, would require a parallel-to-series rearrangement of myosin filaments, which should diminish force and impart plastic behaviour. (B) Continued contraction after stretching of the smooth muscle with long actin filaments, as seen in asthmatic patients, would not require the parallel-to-series rearrangement that is illustrated in (A). In this situation, greater force could be maintained, and the muscle would behave more elastically. 

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WNT and TGF-β cross-talk

In chapter 4 and chapter 5, we also demonstrated that WNT-5A and WNT-11 regulate TGF-β1-induced expression of α-smooth-muscle-actin (α-SMA) via ROCK-mediated actin polymerization. TGF-β is a particularly important cytokine that is involved in various aspects of airway remodelling, most notably fibrogenic responses. TGF-β acts through the very well described canonical pathway that includes phosphorylation and activation of Smad-2 and Smad-3 by the TGF-β receptor 1 (TGFR1). Smad-4 then binds activated Smad-2/3, which enables this complex to translocate to the nucleus and transcribe specific genes. TGF-β can also activate a wide variety of Smad-independent, or non-canonical, pathways, which include TGF-β activated kinase 1 (TAK-1), phosphatidylinositol 3 kinase (PI3K), and Rho family GTPases. TGF-β activation is elevated in asthmatic airways and contributes to airway remodelling through the development of fibrosis. It does so by binding to TGF-β receptors on different cell types that are involved in airway remodelling. In asthma, TGF-β is primarily secreted by eosinophils, but other cell types including airway smooth muscle and airway epithelium also produce the cytokine. TGF-β has been shown to increase proliferation of ASM through the mitogen-activated protein kinase (MAPK) pathway, as well as increase synthesis of extracellular matrix (ECM) proteins. Apart from direct effects on ASM remodelling, TGF-β can modulate ASM proliferation and survival indirectly through the effects of ECM proteins and growth factors. For example, ECM proteins can interact with the ASM through integrins to induce anti-apoptotic effects, which could promote ASM mass as well. TGF-β has been considered a prime candidate for clinical trials in order to alleviate asthmatic airway remodelling, but inhibition of its receptor, TGFR1, with small-molecule compounds have yielded disappointing results. Although these compounds have shown to be efficacious in animal models and in vitro systems, reducing markers of fibrosis as well as ASM proliferation, clinical development of these inhibitors has proven problematic. This was mostly due to adverse effects, so no clinical trials have been conducted within the context of asthma yet. For example, a TGFR1 inhibitor, galunisertib, has been tested in a trial study for cancer, but had significant toxic effects on cardiac function. These adverse effects may be due to the effects of TGF-β on immune responses.
TGF-β family members are involved in the initiation, maintenance, and resolution of inflammatory responses. TGF-β knockout mice develop extensive multi-organ inflammation and die shortly after birth \(^67\), which include chemotaxis for inflammatory cells like neutrophils \(^68\), and suppression of T-cell proliferation and macrophage activation \(^69\). In fact, overexpression of CD4+ T-cell specific TGF-β1 results in suppression of allergic asthma in a murine model \(^70\), and accumulating evidence supports a role for TGF-β in promoting T-regulatory cells and inhibition of T-helper (Th) type 1 and type 2 cells \(^71\). Thus, although it is clear that TGF-β is pivotal in the pathophysiology of asthma, targeting TGF-β directly is not a viable strategy. Inhibiting fibrosis, while maintaining immune function may be better achieved by targeting the downstream targets of TGF-β. In light of this, WNT signalling may be an excellent candidate. In this thesis, we have shown that both WNT-5A and WNT-11 are under control of TGF-β, and that both ligands act redundantly to mediate TGF-β-induced expression of α-SMA. Remarkably, knockdown of WNT-5A or WNT-11 could completely prevent the induction of α-SMA, however, addition of recombinant WNT-5A alone was not sufficient to drive α-SMA expression. These findings suggest that the effects of TGF-β are not simply due to upregulation of WNT ligands. The transcription of the α-SMA gene requires both WNTs and prior stimulation by TGF-β, through an as of yet unknown mechanism. Similar findings have been reported for other TGF-β target genes, like connective tissue growth factor (CTGF) and endothelin-1 \(^72\), although it is worth mentioning here that CTGF and endothelin-1 can drive expression of α-SMA without the presence of TGF-β, and may therefore reflect a different mechanism. Further insight into the underlying mechanisms revealed that de novo synthesis of WNT-5A or WNT-11 feeds back in an autocrine manner to rearrange actin cytoskeletal organization in favour of filamentous actin, through activation of ROCK-I. Actin polymerization releases the actin binding protein and transcriptional activator myocardin-related transcription factor-A (MRTF-A), which translocates to the nucleus to induce expression of α-SMA and modulate bronchial tone. Mechanical forces triggered by bronchoconstriction can result in a feedforward loop, as applied force can activate both TGF-β \(^73\) and RhoA-MRTF-A signalling and subsequent expression of α-SMA \(^74\). The effects of TGF-β-WNT-MRTF-A are not limited to contraction and are relevant for other aspects of
airway remodelling as well. For example, MRTF-A is critically involved in the induction of TGF-β-mediated epithelial-mesenchymal-transition (EMT) \textsuperscript{75,76} and epithelial-to-myofibroblast-transition \textsuperscript{77}. Myofibroblasts are a rich source of ECM proteins, and MRTF-A is an important mediator of myofibroblast activation and expression of ECM proteins \textsuperscript{78}. Inhibition of mechanotransduction by blocking the RhoA-MRTF-A axis attenuates experimental pulmonary fibrosis in mice \textsuperscript{79}. Previous work from our lab has also implicated WNT and TGF-β signalling with ECM production. In airway smooth muscle, \textit{de novo} synthesis of WNT-5A is necessary for the production of ECM proteins mediated by TGF-β \textsuperscript{11}. Here, WNT-5A expression is regulated by TGF-β-activated kinase 1 (TAK1) and requires the transcription factor specificity protein-1 (SP-1) in conjunction with β-catenin \textsuperscript{15}. Thus, human airway smooth muscle displays a substantial degree of cross-regulation between TGF-β and WNT signalling.

Cross-regulation of signalling pathways is becoming an increasingly recognized theme in research. Complete sequences of genomes reveal a remarkably small and conserved set of signalling pathways that account for all biological diversity. It is of no surprise that animals have evolved individual components of signalling pathways to be physically assembled into higher order networks, as it seems incongruous that such a limited set of pathways orchestrates so many diverse cell fates and behaviours. This realization is important also in a clinical setting. In the case of asthma, the cross-regulation between TGF-β and WNT signalling may allow for the development of treatment strategies that can overcome the shortcomings of drugs that target TGF-β signalling more directly (which are associated with severe adverse effects). Going forward, it is essential that we study this level of integration in more detail, as the nature of this cross-talk is generally overwhelmingly complex and highly context-dependent. TGF-β and WNT signalling pathways are intertwined throughout life, and molecularly interact at multiple levels \textsuperscript{80}. Failure to recognize this level of integration will undoubtedly confound the development of effective therapeutic interventions in complex diseases like asthma.

**Cross-regulation in the nucleus mediated by CBP and p300**

Cross-talk between WNT and TGF-β pathways is not limited to secondary
effects mediated by WNT ligands. In the nucleus, a number of modulators exist that direct transcriptional output and are used by WNT and TGF-β effectors, but also other signalling pathways. In chapter 7 and chapter 8 we investigated the role of these nuclear components, focusing specifically on CREB-binding protein (CBP) and E1A-associated protein p300. CBP and p300 are co-factors that are required for transcription factors to carry out their function, usually by allowing access to the transcription initiation site \(^{81,82}\). In eukaryotic cells, DNA wraps around histone octamers to assemble nucleosomes, which are further packed into condensed euchromatin that is inaccessible for transcription \(^{83}\). CBP and p300 can remodel chromatin, which opens its structure and allows for gene transcription to occur \(^{84}\). This is mediated through their intrinsic histone acetyltransferase (HAT) activity \(^{85-87}\). In addition, they facilitate gene transcription by linking DNA-bound transcription factors to the basal transcription machinery and recruit them to the promoter site \(^{88}\). Due to their paralogous nature and sequence similarity, CBP and p300 function in part redundantly. However, in specific circumstances CBP and p300 exert distinct roles \(^{89}\). Both co-factors interact with a wide spectrum of transcription factors \(^{90}\), mainly due to the presence of four recognized transactivation domains (TADs). Many of their interaction partners also have multiple TADs, which together allows for a diverse assembly of different complexes \(^{91,92}\). Interaction with certain transcription factors, but not others, is partly orchestrated through their ability to acetylate non-histone proteins, among which transcription factors, which modulates their activity. Another aspect of selectivity is mediated through the scaffolding actions of β-catenin. Both CBP and p300 can physically interact with the β-catenin region R10-C \(^{93,94}\). In chapter 7 and chapter 8 we have shown that in human ASM, the co-factor/β-catenin interaction is essential in directing not only WNT-responsive gene transcription, but also integrates platelet derived growth factor (PDGF), TGF-β, and NF-κB transcriptional output. These results enforce the idea that individual pathways eventually converge as intertwined signalling modules, that allows cells to read a limited set of extrinsic signals, while being able to mount diverse cellular responses. These findings further strengthen the idea that cross-regulation of different pathways is much more common than we may have believed previously. Based on our current knowledge, it
seems that targeting nuclear components would be a good treatment strategy, as this would avoid a large part of potential interference with other signalling pathways. In light of this, ICG-001, a small-molecule compound that selectively inhibits the β-catenin/CBP interaction (while having no effect on p300) may be an important first step. In chapter 7, we showed that ICG-001 could effectively prevent ASM thickening in a murine model of allergic asthma. Furthermore, in chapter 8 we showed that in human ASM cells, NF-κB-mediated expression of interleukin-6 (IL-6) could be increased or inhibited by targeting the β-catenin/CBP/p300 interaction. PRI-724 is a second generation selective β-catenin/CBP antagonist (IC$_{50}$ 150 nM). It was proven to be safe in pre-clinical drug toxicology studies. A follow-up phase I study on the effects of PRI-724 on solid tumours also showed an acceptable toxicity profile. Additional trials with PRI-724 are underway. Although these developments are exciting, at the same time it is essential that we realize how incredibly complex signal integration in the nucleus is, and that sufficient effort has to be directed towards answering open questions in this regard. For example, WNT/β-catenin signal transduction in the nucleus has revealed itself to be much more complex than was previously thought. Binding of the WNT-responsive transcription factor T-cell factor-4 (TCF-4) occurs in the vicinity of transcription start sites of WNT-target genes. However, at the same time, TCF-4 binding is observed at sites located at great distances from the TSS. Furthermore, at the TSS, TCF-4 clusters around WNT-target genes, binding to intronic, upstream and downstream locations. While some of these binding sites act as classical transcriptional regulatory elements, including regions both upstream and downstream of the gene, the majority of binding sites do not fall under this category. It has been proposed that they may serve to maintain an open chromatin domain, or to provide a local niche to harbour β-catenin. Additionally, many transcription factors, including those of the WNT pathway, contain intrinsically disordered regions (IDRs). IDRs lack a fixed or ordered three-dimensional structure, but are able to undergo binding-coupled folding and adopt multiple conformations, which enables them to interact with a diverse set of binding partners. Many of the TADs of transcription factors are intrinsically disordered, which allow them to bind multiple sites on CBP or p300, as well as other transcription factors. Together,
they may adopt different conformations to accommodate local chromatin structure and the variable distances to the TSS, allowing CBP or p300 to be positioned correctly in order to promote transcription of genes. Furthermore, TADs within CBP or p300 may be occupied by different transcription factors simultaneously, which may provide a mechanism for some transcription factors to modulate the effect of others. These are just a number of examples that hint towards the seemingly promiscuous and highly complex nature of the interactions that take place between co-factors, β-catenin, and transcription factors. A thorough understanding of these events will pave the way for therapeutic interventions that will selectively target aspects of disease, while maintaining cellular homeostasis and integrity.

The ASM as a source of inflammation

As we have already laid out more comprehensively in chapter 1, in the past two decades, the ASM has changed from being regarded simply as a contractile unit that is essential in mediating airway hyperresponsiveness, to being regarded as an important inflammatory cell that can secrete a wide range of cytokines and chemokines. In a disease setting smooth muscle behaves as an important relay, reeling in and harbouring dedicated immune cells to aggravate asthmatic inflammation. At the same time, smooth muscle cells are an important source of pro-inflammatory mediators themselves, that can disrupt tissue homeostasis even without the presence of infiltrating immune cells. These observations have changed significantly how we think about allergic and asthmatic immunology. In chapter 6, we continued with this idea and investigated the effects of smooth-muscle derived WNT-5A in a murine model of allergic asthma. β-catenin-independent WNT signalling, among which WNT-5A, has been linked with a variety of diseases, but little is known how they regulate inflammation in an asthmatic environment. Much more is known about WNT/β-catenin signalling in asthma, which is discussed in detail in chapter 2. For our experiment, we generated a tet-ON smooth-muscle-specific WNT-5A transgenic mouse model, enabling in vivo characterization of smooth-muscle-derived WNT-5A in an allergic asthmatic context, using chronic ovalbumin exposure to drive asthmatic changes. We found that WNT-5A significantly enhanced the production of Th2-
cytokines IL-4, IL-5 and IL-13. In line with this, WNT-5A increased mucous production in ovalbumin-treated animals, and enhanced eosinophilic infiltration and serum IgE. Although a role for Th2 cytokines on smooth muscle function has been clearly described \(^{107}\), the opposite relationship is much less frequently reported. These results therefore highlight the potential of smooth muscle to contribute to allergic inflammation in asthma.

Asthma has been classically considered to be a disease driven by dysregulated Th2 activation, resulting from a Th1/Th2 imbalance \(^{108,109}\). Although this theory been revised over the years, mainly due to observations that Th1 and Th2 cells are both pro-inflammatory and Th1 cells do not always inhibit T cell differentiation to Th2 cells, but can also exacerbate Th2-mediated diseases \(^{110-112}\), the Th2 cell is still an integral part of asthma pathophysiology. Type 2 inflammation can be efficiently suppressed in most patients with asthma with the regular use of glucocorticosteroids, which is the mainstay controller therapy for asthma. However, steroids have multiple local side effects, including dysphonia and candidiasis, as well as systemic side effects, such as cataracts, osteoporosis, and adrenal suppression, especially when high doses are used over prolonged periods of time. The advent of more specific inhibitors, e.g. biologicals targeted against type 2 inflammation, has raised hope that these drugs will provide similar benefits to patients with asthma, while displaying less adverse effects. However, compared to glucocorticoids, these compounds have a more limited effect on airway function and asthma control, even when stratified for different asthma phenotypes or endotypes. Thus, they have so far not been able to replace steroid therapy and are adjunctive at best \(^{113}\). In addition, although Th2-high asthma is generally a corticosteroid-responsive endotype \(^{114-116}\), a notable subgroup of patients with this endotype maintain symptoms and experience severe uncontrolled asthma in spite of regular use of steroids \(^{117-120}\). Novel drug treatment of this group of steroid insensitive patients with severe asthma is highly warranted. Furthermore, some Th2-high asthmatics require high doses of steroids for maintenance therapy, and these patients are in need of alternatives to avoid excessive adverse effects. Collectively, they provide sufficient rationale for the pursuit of novel drug targets for type 2 inflammation in asthma. Our findings laid out in chapter 6 identify WNT-5A as a potential novel target in this regard, as
it seems to specifically affect Th2 immune function. We have not investigated the underlying mechanisms involved in this response, and it is unclear whether the effects of released WNT-5A are due to an immediate effect on dedicated immune cells, or due to secondary effects on the local parenchyma through auto- or paracrine signalling. Nonetheless, our findings that WNT-5A aggravates serum IgE levels and increases IL-5 and IL-13 in the lung, suggests that anti-WNT-5A therapy may affect exacerbation rates in asthma, as this is one of the major effects induced by omalizumab, an anti-IgE biological, as well as by anti-IL-5 and IL-13 therapy. Because IL-5, IL-13 and IgE are mainly mediators of type 2 inflammation, and have very little effect on functional parameters like FEV1, lung function or AHR, it remains to be determined whether anti-WNT-5A treatment would be able to achieve this. However, asthmatic patients treated with anti-IgE or anti-interleukins show evidence of reduced active type 2 inflammation in the lungs, but still exhibit active smooth muscle dysfunction, suggesting that the core physiological abnormalities of excessive tone and AHR in asthma may not necessarily be driven by type 2 inflammation. This is where anti-WNT-5A therapy may confer additional benefit. Due to its effects on actin polymerization in ASM, it is possible that anti-WNT-5A treatment, in addition to reducing markers of type 2 inflammation, would also affect AHR, as we have discussed in chapter 4. Furthermore, as discussed in chapter 2, WNT-5A targets other aspects of innate and adaptive immunity as well. Future efforts have to confirm the usefulness of anti-WNT-5A therapies as a new asthma drug.

**Main conclusions**

- Both the development of and increasing interest in characterizing asthma pheno- and endotypes, and the emerging concept that asthma may have a developmental origin, raises interesting thoughts in terms of future therapy. WNT signalling is increasingly considered to be of extreme relevance in this regard (chapter 2).
- Many physiologically relevant contractile agonists elevate cytosolic calcium or concomitantly increase sensitivity of the contractile machinery to calcium in airway smooth muscle. Efforts that fully describe the cellular mechanisms that mediate these changes (including WNT signalling) are important, as they are integral to fundamental
features of asthma, for example AHR (chapter 3).
- Identifying proteins that influence AHR, but are not part of the contractile machinery is gaining interest in the field. WNT-5A is especially relevant in this regard. While WNT-5A does not induce changes in calcium metabolism, it can increase ASM contraction by reorganizing the actin cytoskeleton in favour of filamentous actin (chapter 4).
- Cross-regulation between biological pathways is crucial to understand how a relatively small set of pathways can orchestrate life. In ASM, WNT and TGF-β signalling are intimately involved, and many of the TGF-β-derived effects are mediated by WNT-5A (chapter 4) or WNT-11 (chapter 5).
- Expression of α-SMA by TGF-β requires WNT-induced actin polymerization through activation of ROCK-I. This releases MRTF-A from the actin pool, which facilitates expression of α-SMA (chapter 4 and chapter 5).
- Although type 2 inflammation in asthmatics with Th2-high asthma can be adequately controlled with corticosteroids in most cases, there is still an unmet need for novel Th2 targets, particularly for patients with severe, steroid insensitive asthma. WNT-5A shows great promise in this regard. In a murine model for allergic asthma, smooth-muscle derived WNT-5A increased the expression of Th2 cytokines, serum IgE levels, and mucous production (chapter 6).
- The β-catenin/CBP interaction is critical for many pathways to mediate their responses, including in ASM. Both proliferation and ECM production require this interaction. Inhibiting it in a murine model for allergic asthma can effectively prevent onset of ASM thickening (chapter 7).
- NF-κB-mediated inflammation is a critical aspect of asthma, and utilizes the catenin/co-factor interaction in many cell types, including ASM. β-catenin is essential in IL-1β-mediated expression of IL-6 by promoting nuclear translocation of the p65 subunit of NF-κB. In the nucleus, CBP and p300 interact with both p65 and β-catenin to direct opposing functions in terms of IL-6 expression (chapter 8).
References


37. Lim, T. K., Pride, N. B. & Ingram, R. H. Effects of volume history during spontaneous and acutely


114. Berry, M. et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-


General discussion