General Introduction
The extracellular matrix

Within the human body, tissue cells reside in a three-dimensional protein network, the extracellular matrix (ECM), which is actively secreted, maintained and molded into the intercellular space by the cells residing in it. The ECM surrounds individual cells and plays a key role in determining physical and mechanical properties of tissues and organs, including the lung. The diverse components that comprise the ECM include collagens, elastin, glycoproteins (including laminins and fibronectin) and proteoglycans (like decorin and biglycan) [1]. In addition to its function as a scaffold, the ECM also influences fluid balance, lung compliance and elasticity and stores inflammatory mediators and growth factors, which may be rapidly released upon cleavage by matrix metalloproteinases (MMPs) and serine proteases [2-4]. Importantly, ECM proteins have also been found to influence cellular functions like migration, differentiation, proliferation and apoptosis.

The extracellular matrix in the airways

Extracellular matrix composition in healthy airways

In the airways, the ECM is part of the cartilage, basement membranes, interstitium and provisional matrices. Cartilage surrounds the (central) airways and prevents airway collapse during breathing [5]. Basement membranes surround the structural cells and separate the cells from the adjacent interstitium [6]. Provisional matrices are formed after tissue injury and are replaced when normal tissue function is restored. The ECM is assembled from a large number of different macromolecules of which the fibrous proteins like collagens and elastin, glycoproteins and proteoglycans are the main components.

Collagens comprise a large family of fibrous ECM proteins and are formed from three polypeptide α-chains coiled in a helical structure [7]. The long, stiff chains contain characteristic repeating -glycine-X-Y-sequences in which the X-position is frequently occupied by proline and the Y-position by 4-hydroxyproline [7]. At least 28 collagen subtypes, divided into 8 subfamilies, are formed in humans (see [7] and [8] for detailed review). Collagens are widespread throughout the body and fulfill a variety of biological functions [7]. In the lung, collagens constitute approximately 15% of total lung dry weight [9]. Collagen IV is most prominently expressed in the basement membranes of the epithelium, vasculature and airway smooth muscle (ASM) [10]. Collagens I, III, V are only weakly expressed in the basement membrane, but are predominantly expressed in the interstitium and beneath the subepithelial basement membrane [10,11]. Collagen fibres are often assembled together with elastic fibres, which are formed from an elastin core surrounded by fibrillin-rich microfibrils, which conveys elastic recoil, whereas the fibrous collagens I and III provide tensile strength [12].

Chapter 1
Laminins are large heterotrimeric glycoproteins comprised of α, β and γ chains, which are important components of basement membranes [13]. Sixteen isoforms, assembled from five laminin α, four β and three γ chains, have been identified [14]. Laminin α2, α3 and α5 chains have been observed in the epithelial basement membranes of healthy subjects. In addition, all β and γ chains are present in the epithelial basement membrane [15-18]. Laminins α4, α5, β1, β2 and γ1 have been found in the basement membrane of ASM cells [15,16,19].

The glycoprotein fibronectin can be found in the circulation in its soluble form and within the ECM in its insoluble form [20]. Expression of fibronectin in tissue is increased during cycles of injury and repair. Accordingly, only diffuse staining of fibronectin has been observed in the airways of healthy subjects [10,21]. Other glycoproteins, like vitronectin, tenascin, thrombospondin or SPARC (Secreted Protein Acidic and Rich in Cysteine) are often increased in tissue undergoing repair as well. However, little is known about their expression in the airways of healthy subjects [1].

Proteoglycans are macromolecules consisting of a core protein with glycosaminoglycan (GAG) side chains, with the exception of hyaluronan which lacks a core protein. GAGs are divided in two classes, the first class being sulphated GAGs, like chondroitin sulphate, heparan sulphate and heparin, and the second class consisting of non-sulphated GAGs, like hyaluronan. The combination of the various core proteins and GAGs results in a large number of proteoglycans of which the small leucine rich proteoglycans (SLRPs) with only limited numbers of GAG side chains, like decorin, biglycan and lumican, and the modular proteoglycans with multiple GAG side chains, like versican, are best characterized [22]. Proteoglycans have been implicated in the assembly of collagen fibrils, the regulation of water balance and the storage of growth factors, chemokines and cytokines [1]. Decorin, biglycan and lumican have been observed in the subepithelial, ASM and adventitial compartments of the airways, with strong staining for decorin and biglycan in the adventitial compartment and within the ASM bundle [23,24]. Staining for versican is mainly observed beneath the epithelial basement membrane and within the airway ASM bundle [23].

Changes in the extracellular matrix in chronic asthma

Asthma is an inflammatory airway disease characterized by airway inflammation, variable airway obstruction and airway hyperresponsiveness (AHR) [25]. AHR is defined by exaggerated airway narrowing in response to a variety of direct (e.g. histamine, methacholine) and indirect (adenosine monophosphate (AMP), fog, cold air, sulphur dioxide and exercise) stimuli [26]. Variable airway hyperresponsiveness occurs after episodic exposure to environmental factors, including allergens, and relates to airway inflammation, whereas persistent airway hyperresponsiveness is considered to reflect structural changes in the airway wall, collectively termed airway remodelling [27,28]. The most striking changes in the structure of the airway wall of asthmatics include epithelial...
shedding, increased ASM mass, goblet cell hyperplasia and metaplasia, increased microvasculature, subepithelial thickening and alterations in the ECM composition [29-31]. Mathematical modelling studies have indicated that increased ASM mass is likely to be the most important factor contributing to persistent airway hyperresponsiveness [32,33]. On the other hand, increased ECM deposition beneath the epithelium and within the ASM layer could be protective against airway constriction, as it may stiffen the tissue as a result of decreased elasticity and increased preload, whereas increased ECM deposition in the adventitia may lead to enhanced airway narrowing due to uncoupling of the tissue from the elastic recoil of the surrounding tissue [34].

Studies of endobronchial and post-mortem biopsies on the nature of the subepithelial alterations in the ECM in asthmatics have revealed increased deposition of collagen I, III and V, fibronectin, tenascin, hyaluronan, versican, biglycan, lumican and several laminin chains, including α2, α3, α5, β1, β2 and γ1 chains [10,15,21,35-39]. Although weak, staining of laminin α1 chains has also been observed in the airways of allergic asthmatics, while no expression was observed in the airways of non-allergic asthmatics or healthy subjects [39]. In addition, both in allergic and non-allergic asthmatics with compromised epithelial integrity, increased laminin γ2 deposition has been observed, which correlated closely with the level of epithelial integrity [39]. Expression of collagen IV, decorin and elastin, on the other hand, was decreased in the airway wall of asthmatics [1,40].

Post-mortem studies on airway tissue from individuals with fatal asthma have indicated that increased ECM deposition is not only present beneath the epithelium, but also inside and outside of the ASM bundle [41]. Increased ECM expression in the ASM bundle has been reported to involve deposition of collagen type I, fibronectin, hyaluronan, versican, biglycan, lumican and elastic fibres [24,42-44]. Increased collagen I and versican deposition was, however, not found in a subsequent study [44]. AHR may be linked to changed ECM deposition as it was shown that in asthmatics, airway responsiveness to methacholine was inversely correlated with elastin expression in the ASM bundle [45].

Mechanisms regulating extracellular matrix composition

Tissue and ECM turnover are physiological processes, which are dynamically governed by a balance between matrix synthesis and matrix degradation [9]. In the airways, matrix turnover is estimated to amount more than 10% per day [46]. ECM turnover may be increased in asthmatics, as levels of cellular fibronectin, laminin degradation products and hyaluronan are increased in the bronchoalveolar lavage (BAL) fluid [47-49], of which the hyaluronan levels were correlated with asthma severity [48].
Epithelial cells and fibroblasts are considered to be the main source of ECM in the airways. However, ASM cells also produce a large variety of ECM proteins, including collagens, fibronectin, laminins, perlecan, elastin, thrombospondin, versican, decorin and hyaluronan [50-53]. Asthmatic and healthy ASM produce different ECM profiles, as indicated by increased production of collagen I, perlecan and fibronectin, and decreased production of laminin α1, chondroitin sulphate, collagen IV and hyaluronan by asthmatic ASM [50,53,54]. Production of ECM proteins is increased by profibrotic factors like transforming growth factor-β (TGF-β), granulocyte macrophage colony-stimulating factor (GM-CSF), connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF) [55]. Moreover, asthmatic ASM cells produce more CTGF in response to TGF-β [56], suggesting that this factor may be involved in changed ECM production by these cells. In addition, exposure of healthy ASM cells to atopic serum increases the production of fibronectin, laminin γ1, perlecan and chondroitin sulphate [57].

Degradation of ECM is governed by a variety of proteases and binding proteins, of which the matrix metalloproteinases (MMPs), their inhibitors – the tissue inhibitors of MMPs (TIMPs) – and A disintegrin and metalloproteinases (ADAMs) are best recognized [58,59]. Several studies have shown that in asthma, expression of MMP-2, MMP-3 and MMP-9 is increased, of which MMP-9 is increased most predominantly (reviewed in [59]). In addition, polymorphisms in the ADAM33 gene correlate with asthma incidence, bronchial hyperresponsiveness and lung function decline, although the precise mechanism remains to be determined [60,61].

Extracellular matrix proteins, integrins and airway smooth muscle function

*Extracellular matrix proteins affect airway smooth muscle function*

Increased ASM mass is, next to increased and changed ECM deposition, another hallmark of airway remodelling in asthmatics [62-64]. Increased ASM mass may involve increased cell numbers (hyperplasia), cell size (hypertrophy) or a combination of both [62-64]. ASM hyperplasia may, at least partly, be explained by enhanced proliferative responses and in culture ASM cells derived from asthmatics proliferate faster than those obtained from healthy subjects [65,66]. In addition to increased proliferative capabilities, contractile function and synthetic capabilities of ASM cells derived from asthmatics proliferate faster than those obtained from healthy subjects [65,66]. In addition to increased proliferative capabilities, contractile function and synthetic capabilities of ASM cells derived from asthmatics have also been shown to be increased [54,56,67]. ASM may exert these functions as they retain the ability of reversible phenotypic plasticity, enabling them to switch between proliferative, synthetic, contractile and migratory phenotypes [68,69]. In vitro, exposure of ASM cells to mitogens results in the switch from a contractile to a proliferative phenotype, associated with a decreased contractile ability [70] due to decreased expression of contractile proteins [68]. Conversely, removal of
growth factors, in the presence of insulin or TGF-β, results in the reintroduction of a (hyper)contractile phenotype [71, 72]. For a detailed review of the literature on ASM phenotype plasticity, the reader is referred to references [72] and [73].

Various studies have addressed the effects of ECM proteins on ASM function, including ASM proliferation, contractile protein expression, maturation, synthetic function, survival and migration (Figure 1). Of the collagens, collagen type I has been most extensively studied. Monomeric collagen I has been shown to enhance basal and growth factor-induced ASM proliferation, ASM survival and synthesis of pro-inflammatory mediators like eotaxin, RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted, CCL5) and GM-CSF [54, 74–77]. Collagen I concentration-dependently increases ASM migration [78]. The reduction in contractile protein expression induced by platelet-derived growth factor (PDGF) is further reduced by monomeric collagen type I [75]. Surprisingly, although the fibrillar form of collagen I increased cytokine synthesis to a similar extent as did monomeric collagen I, no effects of fibrillar collagen I were observed on ASM cell proliferation [54, 77], whereas recently fibrillar collagen I has even been shown to inhibit both basal and growth factor-induced proliferation [79]. In addition, inhibition of collagen degradation by the MMP inhibitor ilomastat further enhanced the growth-attenuating effects of fibrillar collagen I, indicating that degradation of collagen to its monomeric isoform may enhance ASM proliferation [79]. Little is known on the effects of other collagens on ASM function. The studies available indicate that culturing ASM cells on collagen type III does not change PDGF-induced ASM cell proliferation [77], but increases PDGF-induced migration [78]. Collagen type V increased migration, but was without effects on ASM cell survival [76, 78]. Finally, collagen IV has been shown to inhibit ASM cell apoptosis [76].

Many effects observed for fibronectin are comparable to those observed for monomeric collagen I. Growth factor-induced proliferation, cytokine synthesis and survival are increased in cells cultured on fibronectin matrices and the effects of PDGF on contractile protein expression are enhanced [54, 74–77]. In addition, fibronectin increased migration of ASM cells towards PDGF [78]. Vitronectin also increased growth factor-induced proliferation, although to a lesser extent than observed for fibronectin [77]. No effects of vitronectin were observed on ASM survival [76].
Laminin-111 (laminin-1) or matrigel, a basement membrane extract containing multiple ECM components including laminins [80], reduced growth factor-induced proliferation and prevented growth factor-induced reductions in contractile protein expression [75]. Laminin-111 also increased ASM survival [76]. Moreover, maturation of ASM cells to a contractile phenotype – in the presence of insulin – is accompanied by an increased expression of laminin α2, β1 and γ1 chains, found in laminin-211 (laminin-2). Increased expression of these laminin chains was required for the maturation as contractile protein accumulation by serum deprivation is normalized by the laminin competing peptide Tyr-Ile-Gly-Ser-Arg (YIGSR) and the Arg-Gly-Asp (RGD)-containing peptide Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) [81].

Proteoglycans differentially affect ASM function. Culturing ASM cells on decorin decreased PDGF-induced proliferation and increased apoptotic rates, while biglycan did not have an effect on both parameters [82]. Decorin may also play an important role in ASM maturation by binding of and inhibiting the function
of TGF-β1 [83,84]. Heparin, heparan and chondroitin, other members of the proteoglycan family, also inhibited serum-induced ASM proliferation [85].

Changes in the ECM profile produced by asthmatic ASM may contribute significantly to the changed function of asthmatic ASM cells [50,54]. Both enhanced proliferation and increased eotaxin synthesis by asthmatic ASM cell is dependent on the ECM: healthy ASM cells cultured on an ECM laid down by asthmatic ASM cells, showed increased proliferative rates and increased synthetic capacities and vice versa [50,54], suggesting that in asthma the ECM may be a critical regulator of increased ASM mass and persistent inflammation in the remodelled airway.

**Integrins and airway smooth muscle function**

Cells interact with their surrounding matrix mainly through integrins, a group of heterodimeric transmembrane glycoproteins, which interact with specific sequences within the ECM proteins. Eighteen α integrin subunits and eight β integrin subunits forming twenty-four heterodimers have been identified thus far [86]. In culture, ASM cells have been shown to express the α1, α2, α3A, α4, α5, α6A, α6B, α7B, αν, αvβ3 and β1 subunits, while the α3B, α7A, β2 and β4 subunits are relatively rare (Table 1)[76,77,87,88].

The majority of the studies on the role of integrins in ECM-induced changes in ASM function have focused on collagens, fibronectin and laminins. Increased synthetic responses of ASM cells in response to IL-1β or IL-13 on collagen I matrices required interaction with the collagen binding integrin α2β1 [54,87]. Similarly, enhancement of growth factor-induced proliferation by monomeric collagen I was inhibited by α2β1 function-blocking antibodies. In addition, blocking of the fibronectin binding integrins α4β1 and α5β1 also prevented the enhancement of growth factor-induced proliferation by monomeric collagen I, suggesting that fibronectin may also be involved in the enhanced proliferation [77]. Attachment of ASM cells to collagen I required the α2β1 integrin and the fibronectin binding integrin αvβ3, however [77].

Similar to collagen I, enhancement of growth factor-induced proliferation by fibronectin required interaction with the α2β1, α4β1 and α5β1 integrins [77]. The collagen binding integrin α2β1 and the fibronectin binding integrins α5β1, αvβ1 and αvβ3 are important in the increased eotaxin production in response to IL-1β by ASM cells cultured on fibronectin [87]. Moreover, peptides containing the RGD peptide sequence, present in the integrin recognition site of several ECM proteins [92,93], also inhibited the enhancement of IL-1β-induced eotaxin release [87]. Enhancement of IL-13-induced eotaxin release, on the other hand, only required interaction with the α5β1 integrin [54]. Attachment of ASM cells to fibronectin is also mediated by α5β1 integrins [77].
Table 1: Airway smooth muscle: integrin expression and function.

<table>
<thead>
<tr>
<th>Integrin</th>
<th>Expression level</th>
<th>Known functions in ASM</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1</td>
<td>~30-50%</td>
<td>~</td>
</tr>
<tr>
<td>α2</td>
<td>~60-80%</td>
<td>Enhanced growth factor-induced proliferation on collagen I and fibronectin matrices [77], enhanced cytokine release on collagen I and fibronectin matrices [87], attachment to collagen I [77], resistance to glucocorticoid action on collagen I matrices [89].</td>
</tr>
<tr>
<td>α3A</td>
<td>~50%</td>
<td>Increased expression by PDGF stimulation [77], increased expression during maturation [88], inhibition of α5β1 expression [88].</td>
</tr>
<tr>
<td>α3B</td>
<td>fraction</td>
<td>~</td>
</tr>
<tr>
<td>α4</td>
<td>~20-30%</td>
<td>Enhanced growth factor-induced proliferation on collagen I and fibronectin matrices [77], serum-induced proliferation of nonasthmatic ASM [90].</td>
</tr>
<tr>
<td>α5</td>
<td>~100%</td>
<td>Enhanced growth factor-induced proliferation on collagen I and fibronectin [77], serum-induced proliferation of nonasthmatic and asthmatic ASM [91], attachment to fibronectin [77], ASM survival [76], cytokine release on fibronectin and increased cytokine release by asthmatic ASM [54,87], regulation of fibronectin expression [91].</td>
</tr>
<tr>
<td>α6A</td>
<td>~40%</td>
<td>Increased expression during maturation [88], negative regulation of α5β1 expression [88]</td>
</tr>
<tr>
<td>α6B</td>
<td>~30%</td>
<td>~</td>
</tr>
<tr>
<td>α7A</td>
<td>fraction</td>
<td>~</td>
</tr>
<tr>
<td>α7B</td>
<td>~20%</td>
<td>Increased expression during maturation [88], required for maturation [88]</td>
</tr>
<tr>
<td>αv</td>
<td>~50-100%</td>
<td>Enhanced cytokine release on fibronectin [87], serum-induced proliferation [90]</td>
</tr>
<tr>
<td>αvβ3</td>
<td>~50%</td>
<td>Attachment to collagen I [77], enhanced cytokine release on fibronectin matrices [87]</td>
</tr>
</tbody>
</table>

During maturation, ASM cells not only increase the expression of contractile marker proteins and laminin α2, β1 and γ1 chains [81], but also the expression of the laminin binding integrin subunits α3A, α6A and α7B [88]. Increased expression of the α7 subunits was shown to be restricted to cells acquiring a contractile phenotype. Knockdown of the α7 integrin, but not of the α3 or α6 integrins, fully prevented phenotype maturation, indicating an essential role for this integrin in ASM maturation. Interestingly, knockout of the laminin binding α3 or α6 integrins increased expression of the fibronectin α5 integrin [88], suggesting an inverse relationship between laminin-binding and fibronectin-binding integrins.
Integrins of the α5β1 subtype have been implicated in a number of ASM functions. Survival of ASM cells on several matrices is significantly reduced in the presence of α5β1 integrin blocking-antibodies [76]. Moreover, survival of ASM cells was also attenuated in the presence of RGD containing peptides [76]. In addition, α5β1 integrins are important regulators of fibronectin expression and of serum-induced proliferation of both asthmatic and non-asthmatic ASM cells [91]. Preliminary results also indicate that serum-induced proliferation of non-asthmatic ASM cells requires α5β1 and α4β1 integrins, whereas asthmatic ASM cells are unresponsive to inhibition of α4β1 integrins [90]. Moreover, increased eotaxin release by asthmatic ASM is significantly inhibited by antibodies blocking the α5β1 integrin [54].

**Integrin-induced signalling**

Integrins not only provide a physical link between the ECM and intracellular compartment, but may also trigger a large number of intracellular signalling cascades to influence cellular processes, including proliferation, differentiation, migration and apoptosis. Many of these signalling pathways are also activated by receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs) [94-96].

The biologically active sites in the ECM interacting with the integrins, like for example the RGD binding site in fibronectin, are usually not exposed in mature ECM proteins, but may become exposed after structural or conformational changes (for a detailed review on matricryptic sites see [97]). Many integrins are not constitutively active. However, upon activation, integrins mediate signal transduction through the cell membrane in both directions: binding of ECM proteins to the extracellular domain of integrins elicits signalling cascades in the cell, via the cytoplasmic domains of the integrin (outside-in signalling), whereas the binding between the integrins and the ECM can be activated or enhanced from the inside of the cell (inside-out signalling) [86].

Intracellularly, integrins activate various protein tyrosine kinases, including integrin linked kinase (ILK) and focal adhesion kinase (FAK) [98]. ILK may be activated by beta integrin subunits and has been shown to be important in the expression of ASM contractile proteins. Knock-down of ILK increased gene expression of a number of contractile proteins, including smooth muscle specific myosin heavy chain (sm-MHC) SM22α and calponin, but only increased protein expression of sm-MHC. This increase was associated with a decreased phosphorylation of protein kinase B (PKB/Akt) and increased binding of serum response factor (SRF) to the promoter for the sm-MHC gene. Conversely, overexpression of ILK had the opposite effect on these processes [99].

Activation of FAK is initiated by autophosphorylation of tyrosine 397 (Y397) [94]. Autophosphorylation requires clustering of the integrins, which may occur after binding to the ECM. The integrin clustering triggers a conformational
change in the associated FAK that alters the interaction of the FERM domain with the kinase domain [100]. In addition to activation of FAK by integrins, FAK may also be phosphorylated by growth factors receptors and G protein-coupled receptors. Growth factor receptors activate FAK via interaction with the FERM domain [101], whereas G protein-coupled receptors activate FAK via a mechanism which is currently unclear, but appears to involve Rho-dependent signalling pathways [96]. Phosphorylation of Y397 generates a high-affinity binding site for Src (Rous sarcoma oncogene cellular homolog) and results in the recruitment and binding of cellular Src to pY397 [102]. Cellular Src subsequently phosphorylates Y576/Y577 in the kinase domain of FAK, which is essential for maximal FAK kinase activity and activation [103]. Studies on the role of FAK in tracheal smooth muscle have indicated that phosphorylation and activation of FAK can be increased by mechanical strain [104] and by acetylcholine (ACh), in a Ca²⁺-independent fashion [105]. Stimulation with ACh also increased the membrane localization of FAK and of cytoskeletal linker proteins like paxillin, vinculin, talin and α-actinin [106]. Depletion of FAK from the tracheal tissue decreased KCl- and ACh-induced contraction in tension, myosin light chain phosphorylation and increase in intracellular Ca²⁺, indicating that FAK plays an important role in ASM contraction [107].

In addition to its role in ASM contraction, activation of FAK is also important in proliferation of various cell types [103]. Upon autophosphorylation and activation, FAK activates multiple signalling cascades, including the phosphatidylinositol 3-kinase (PI3-kinase) and extracellular signal-regulated kinase (ERK) signalling pathways [94]. Although the contribution of these signalling cascades to integrin-mediated changes in ASM function are currently unknown, activation of the PI3-kinase and the ERK signalling pathways has been shown to be critical in the response of ASM cells to peptide growth factors [95]. Thus, activation of PI3-kinases is important in growth factor-induced ASM cell proliferation and hypertrophy [108-110]. Activation of the PI3-kinase is associated with transcriptional activation and protein synthesis leading to ASM cell proliferation and hypertrophy [108,110]. ERK1/2 (or p42/p44 mitogen activated protein kinases (MAPKs)) are involved in the transfer of growth promoting signals to the nucleus and the subsequent induction of ASM proliferation [111]. In addition to p42/p44 MAPKs, p38 MAPK, another member of the MAPK family, is also involved in the regulation of growth factor-induced proliferation of ASM cells [112]. Collectively, these observations suggest that activation of PI3-kinase and MAPK pathways by FAK may contribute to ECM-induced changes in ASM function (Figure 2).
Figure 2: Proposed mechanism by which integrins may activate intracellular signalling cascades and regulate ASM phenotype. Clustering of integrins (ITG) in response to ECM proteins triggers a conformational change in the focal adhesion kinase (FAK), resulting in an autophosphorylation at Y397. In addition, FAK may be activated by receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCR) as well. Phosphorylation of FAK on Y397 creates a binding site for Src, which in turn phosphorylates FAK on Y576/Y577, leading to the full activation of the kinase, which may then activate PI3-kinase (PI3K) and mitogen activated protein kinase (MAPK) signalling pathways to regulate ASM phenotype and function. In addition, integrins may activate integrin-linked kinase (ILK) and protein kinase B (PKB/Akt), which inhibit ASM contractile protein expression. See text for detailed description.

Effects of extracellular matrix proteins on airway smooth muscle response to respiratory therapeutics

ECM proteins not only directly affect ASM function, but also affect the response of ASM towards asthma therapeutics. Studies by Freyer et al, have indicated that changes in the ECM environment may affect the response of ASM cells to \( \beta_2 \)-adrenoceptor agonists, used for the relief of acute bronchospasm [113,114]. Thus, it was shown that production of the second messenger cAMP in response to \( \beta_2 \)-adrenoceptor stimulation was increased in cells cultured on fibronectin,
whereas collagen V and laminin-111 decreased cAMP accumulation. No effects of collagen I or collagen IV on cAMP accumulation were observed [113]. These changes were due to differences in Gαi activity but not Gαq protein expression, which causes inhibition of adenylyl cyclase activity [113].

Effects of glucocorticosteroids, used for the control of (chronic) asthma symptoms [115], have been investigated on ECM production and proliferation of ASM cells. Production of ECM proteins by ASM cells appears to be insensitive to glucocorticoids and treatment may even increase ECM production [116]. The increased ECM deposition by ASM cells in response to healthy or atopic serum is not affected by treatment with beclomethasone, although treatment did inhibit the increases in ASM cell number [57]. Treatment with beclomethasone even increased production of fibronectin, perlecan and chondroitin sulphate and this increase was larger in cells exposed to atopic serum [57]. In ASM cells and intact bronchial rings, treatment with β2-agonists or glucocorticosteroids did not inhibit TGF-β-induced increases in collagen I, fibronectin or CTGF, whereas the PDE4 inhibitor roflumilast did. Moreover, in the absence of TGF-β glucocorticosteroids increased expression of these proteins [117].

Glucocorticoids have been shown to inhibit human ASM cell proliferation [118-120]. Inhibition of human ASM cell proliferation required downregulation of cyclin D1 and reduced phosphorylation of retinoblastoma protein (pRb)[120]. In human ASM cells cultured on collagen I, however, inhibition of basic fibroblast growth factor (bFGF)-induced proliferation by glucocorticoids is largely abrogated [89,121]. This resistance to glucocorticoid action was no longer observed in the presence of α2β1 function blocking antibodies [89], indicating that this integrin is importantly involved in this process. By contrast, production of the cytokine GM-CSF by human ASM was inhibited by the glucocorticoid dexamethasone independent of the ECM environment, suggesting that proliferation and synthetic functions are differentially regulated by glucocorticoids [121]. On the other hand, no effects of collagen I were observed on glucocorticoid sensitivity in bovine ASM cell proliferation [74]. The β2-agonist salbutamol inhibited bFGF-induced human ASM cell proliferation in cells cultured on both laminin and collagen I, whereas GM-CSF release was not inhibited by salbutamol in cells cultured on collagen I [122]. Collectively, these findings suggest that changes in the ECM deposition in the airway wall, especially in the ASM surroundings, may change the effectiveness of the medication in asthma.

**Aims of the studies**
The above mentioned observations suggest that ECM proteins and their integrins may be important mediators of altered ASM function as observed in asthma. The primary aim of this thesis was to investigate the regulation of ASM phenotype and function by ECM proteins and to investigate the potential contribution of these effects to ASM remodelling in asthma.
Chapter 2 provides a comprehensive review of the abnormalities observed in asthmatic ASM function. In addition, the mechanisms by which structural components of the airway wall may contribute to abnormal ASM behaviour in asthma are discussed.

Chapters 3 and 4 address the effects of different ECM proteins on ASM phenotype and function. Chapter 3 describes the effects of prolonged treatment with the ECM proteins collagen type I, fibronectin and laminin-111 on contractility of intact bovine tracheal smooth muscle (BTSM) tissue. In addition, these effects were related to contractile protein expression and cell proliferation, in order to assess phenotype modulation under these conditions. Moreover, the effect of these ECM proteins on PDGF-induced phenotype modulation was investigated as well. In chapter 4, some of the findings observed in BTSM were translated to human tracheal smooth muscle (HTSM). To this aim, the effects of prolonged treatment of intact HTSM strips with collagen I, in the absence and presence of PDGF, were investigated on contractility, contractile protein expression and cell proliferation.

Chapter 5 describes the role of laminins in the induction of a hypercontractile ASM phenotype. Previous studies have indicated that long-term exposure of BTSM to insulin induces a functionally hypoproliferative phenotype [71,123]. The contribution of laminins to the induction of such a hypercontractile, hypoproliferative phenotype was assessed, using the laminin competing peptides Tyr-Ile-Gly-Ser-Arg (YIGSR) and Arg-Gly-Asp-Ser (RGDS). In addition, the effects of insulin on laminin mRNA and protein expression were investigated, as well as the potential signalling mechanisms involved (PI3-kinase and Rho kinase).

The potential contributions of ECM proteins and their integrins to airway wall remodelling in vivo were assessed using a guinea pig model of chronic allergic asthma [124]. Chapter 6 describes the effects of the integrin blocking peptide RGDS on parameters of airway remodelling induced by repeated allergen-challenge. Thus, the contribution of RGD-binding integrins to allergen-induced ASM hyperplasia, increased contractile protein expression and hypercontractility as well as inflammation and fibrosis were assessed. Furthermore, to investigate potential underlying mechanisms the effects of RGDS on ECM- and growth factor-induced proliferation and maturation of human ASM cells were assessed in vitro. The effects of the specific laminin competing peptide YIGSR on airway remodelling in vivo are described in Chapter 7.

The involvement of pro-mitogenic signalling pathways in collagen I-induced changes of ASM phenotype was studied in Chapter 8. The role of FAK in ASM cell adhesion and in collagen I-induced cell proliferation was assessed by overexpression of the kinase as well as by overexpression of two FAK inhibitors.
The contribution of the downstream signalling pathways Src, PI3 kinase, p42/p44 MAPK and p38 MAPK to the induction of a proliferative, hypocontractile ASM phenotype by collagen I was investigated using specific inhibitors.

Finally, in Chapter 9 the functional impact of glucocorticoids and $\beta_2$-adrenoceptor agonists on ASM phenotype switching was studied. The effects of the glucocorticosteroids fluticasone, budesonide and dexamethasone and the $\beta_2$-adrenoceptor agonist fenoterol on the induction of a proliferative, hypocontractile BTSM phenotype by PDGF or collagen I were assessed. As previous studies have indicated that glucocorticosteroids and $\beta_2$-adrenoceptor agonists synergize to inhibit ASM cell proliferation [119], the functional impact of this synergism on the induction of a proliferative, hypocontractile phenotype was assessed as well.

References

Chapter 1


55. Burgess JK. The role of the extracellular matrix and specific growth factors in the regulation of inflammation and remodelling in asthma. *Pharmacol Ther* 2009.


