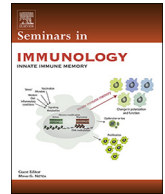




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Immune induction of airway remodeling

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ABSTRACT

Airway remodeling is accepted to be a determining component within the natural history of asthma. It is a phenomenon characterized by changes in the airways structures that marches in parallel with and can be influenced by airway inflammation, floating at the interface between both natural and adaptive immunity and physical and mechanical cells behavior. In this review we aimed to highlight the comprehensive, yet not exhaustive, evidences of how immune cells induce, regulate and adapt to the recognized markers of airway remodeling.

Mucous cell hyperplasia, epithelial dysfunction and mesenchymal transition, extracellular matrix protein synthesis and restructuration, fibroblast to myofibroblast transition, airway smooth muscle proliferation, bioactive and contractile properties, and vascular remodeling encompass complex physiopathological mechanisms that can be induced, suppressed or regulated by different cellular and molecular pathways. Growth factors, cytokines, chemokines and adhesion molecules expressed or derived either from the immune network of cells infiltrating the asthmatic airways and involving T helper lymphocytes, immune lymphoid cells, dendritic cells, eosinophils, neutrophils, mast cells or by the structural components such as epithelial cells, fibroblasts, myocytes, airway smooth muscle cells concur with protein cellular matrix component and metalloproteinases in modifying the airway structure in a detrimental way. The consequences in lung function decline, fixed airway obstruction and clinical severity of the disease suggest the possibility of identify among the immune molecular pathway of remodeling some biological parameters or signal pathway to be either a good tracer for monitoring the disease evolution or a target for hypothetical phenotypes and endotypes. In the era of personalized medicine, a biomarker of remodeling might predict a response to small-molecule inhibitors or biologicals potentially targeting a fundamental aspect of asthma pathogenesis that impacts on the low responsiveness to airway inflammation directed treatments.

Abbreviations: BKca, Activated K channels; AECs, Airway epithelial cells; AHR, Airway hyperresponsiveness; ASMc, Airways smooth muscle cells; alpha-SMA, Alpha-smooth muscle actin; AREG, Amphiregulin; BMPs, Bone morphogenetic proteins; BDNF, Brain-derived neurotrophic factor; BALF, Bronchoalveolar lavage fluid; CDHR3, Cadherin Related Family Member 3; CCR, C-C chemokine receptor; CAMs, Cellular adhesion molecules; CXCL, C-X-C motif ligand; CXCR, C-Chemokine receptor; CHI3L1 / YKL-40, Chitinase-3-like protein 1; CLP, Chitinases-like proteins; CTGF, Connective Tissue Growth Factor; Cx43, Connexin 43; DI, Deep inspiration; DDP-4, Dipeptidyl-peptidase-4; ADAM33, Disintegrin and metalloproteinase domain-containing protein 33; EPC, Endothelial progenitor cells; EGF, Epithelial growth factor; EMTU, Epithelial-mesenchymal trophic unit; EMT, Epithelial-to-mesenchymal transition; ECMs, Extracellular matrix proteins; ERKs, extracellular signal-regulated kinases; FGF, Fibroblast growth factor; FEV1, Forced expiratory volume in the first second; FENO, Fractional exhaled nitric oxide; Gal-3, Galactin-3; GABA, gamma-Aminobutyric acid; GATA-3, GATA binding protein 3; GM-CSG, Granulocyte-macrophage-colony stimulating factor; HDM, House dust mite; IP3, Inositol triphosphate; IL, Interleukin; ST2, interleukin-1 receptor family member; JAK, Janus kinase; LPA, Lysophosphatidic acid; MCs, Mast cells; MMP, Matrix metalloproteinase; MSCs, Mesenchymal stromal cells; MAPK, mitogen-activated protein kinase; MCP, Monocyte chemoattractant protein; MUC5B, mucin-5B; NGF, Nerve growth factor; Abl, Non-receptor tyrosine kinase; OPN, Osteopontin; OGR1, Ovarian cancer G-protein coupled receptor 1; OVA, Ovalbumin; PARR, Pattern recognition receptors; PDGF, Platelet-derived growth factor; Col1a2, proCollagen1a2; PGE2, Prostaglandin E2; EP, Prostanoid; GRKs, protein-coupled receptor kinases; GPCRs, protein-coupled receptors; PGs, Proteoglycans; PMVECs, Pulmonary microvascular endothelial cells; RTKs, Receptor tyrosine kinases; RANTES, Regulated upon Activation, Normal T cell Expressed, and Secreted; RBM, Reticular basement membrane; BMPs, Selected bone morphogenetic proteins; STAT, Signal transducer and activator of transcription; SLC, Small latent complex; SCF, Stem-cell factor; STIM1, Stromal Interaction Molecule 1; TAS2R, Taste 2 receptor protein; LTBP, TGF- β binding proteins; TSLP, Thymic stromal lymphopoietin; TIMPs, tissue inhibitors of metalloproteinases; TLR, Toll-like receptor; TGF- β , Transforming growth factor-beta; ILC2s, Type 2 innate lymphoid cells; Fc ϵ RI, Type I High affinity receptor for IgE; CLRs, C-type lectin receptors; VEGF, Vascular endothelial growth factor; WNT5A, Wnt Family Member 5A; CXCL, D-X-C motif chemokine

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1. Introduction

Immunologic factors play a pivotal role in the development of asthma and airways diseases. Persistent airways inflammation is a key feature of asthma that is regulated by both the innate and adaptive arms of the immune system. Throughout the natural history of the disease airway inflammation is accompanied by structural changes of the walls defined as remodeling. [1] Actually asthma and allergic inflammation are the results of a complex interplay among inflammatory cells including eosinophils, neutrophils, mast cells, lymphocytes, dendritic and innate lymphoid cells and structural tissue cells such as epithelial and smooth muscle cells, fibroblasts and endothelial cells.

Whether remodeling follows the inflammatory effects as the result of repairing mechanisms or represents an independent phenomenon is a contention sustained by different arguments. [2]

2. Definition and histopathological features of airway remodeling

Remodeling consists of structural alterations of the tissues of the wall airways. In asthma structural changes occur primarily in the major bronchi and along with the disease development involve smaller bronchi and bronchioles. Wall thickening is the main features of remodeling and involves all the components: the epithelium, the basement membrane (BM), the subepithelial layer and the smooth muscle. [3]

The epithelium of the bronchi appears injured, often friable and disintegrating and epithelial shedding is the prominent feature. Airway epithelial cells (AECs) appear hyperplastic, in particular in the component of the goblet cells and they lose their cilia. The result of these alterations is the breakdown in epithelial tight junction integrity and impaired repair after injury [4] as well as upregulated mucin synthesis.

Thickening of the lamina *reticularis*, named reticular basement membrane (RBM), is a hallmark of airways remodeling. It consists of deposition of extracellular matrix proteins (ECMs), mainly collagen, fibronectin and proteoglycans (PGs). It has been observed that particularly collagen I, laminin and tenascin have a stronger staining density in the RBM of airway wall biopsies in asthma [5], and this seems more noticeable in severe asthma [6]. On the contrary, the true basement membrane (lamina *rara* and lamina *densa*), composed mainly by collagen IV, is not increased in thickness in asthmatic subjects. ECMs are produced by fibroblasts and myofibroblasts under the stimulus of epithelial cells. This pathway signaling is called epithelial-mesenchymal trophic unit (EMTU) [7]. Within the submucosal layer are also localized inflammatory cells such as mast cells (MCs), eosinophils and T lymphocytes: ECM proteins might also influence cellular function, including adhesion, differentiation, and survival.

Both hyperplasia (cell proliferation) and hypertrophy (increase in size) of airways smooth muscle cells (ASMC) in asthma remodeling is a key process [8]. Changes in ASM cell contractility have been described contributing to the pathophysiology of airway hyperresponsiveness (AHR) and airway obstruction. ASM cells not only are increased in mass but are biologically active participating in the synthesis of ECMs expressing cellular adhesion molecules (CAMs) and receptors in response to growth factors, cytokines and chemokines derived from epithelial and inflammatory cells. Eventually, ASMC can migrate toward the epithelium.

In the space between the muscle layer and the surrounding parenchyma, an abnormal increase in the number and size of blood vessels has been observed. Angiogenesis in this context causes increased microvascular permeability, mediated by vascular endothelial growth factor (VEGF), oedema and subsequent thickening of airways walls in asthmatic patients. New blood vessels favor the arrival of inflammatory cells. [9]

3. Consequences of remodeling on airway function in asthma

The main consequences of the structural changes of remodeling are alteration in barrier function, lack of endogenous bronchodilators, mucous secretions, increased airway narrowing and alteration in tissue and mechanical properties. These alterations in the airways cause a detrimental effect on lung function. First the development of decline of lung function over time. Second the contribution to progressive fixed and irreversible airway obstruction. Other effects of airway remodeling are reduction of airway distensibility and bronchodilator response, loss of protective smooth muscle stretch relaxation, loss of lung elastic recoil. On the other hand development and persistence of AHR is dependent on airway remodeling events, including collagen deposition and increase in ASM mass and contractile capacity [10]. The relationship between AHR and remodeling is however controversial and, at least in severe asthma, an inverted correlation exists between reactivity to methacholine and airway wall thickening that can be explained by alteration of mechanical properties of ASM and the role of MCs located in the ASM bundles.

4. Inflammation and remodeling

Within the classical description of the immediate and late inflammatory response in asthma and allergic airways diseases, Type 2 T helper lymphocytes (Th2) represent the inevitable crossing point for cell recruitment and activation as well IgE antibodies synthesis. Moreover, the Th2-high phenotype, characterized by the release of signature cytokines interleukin IL-4, IL-5, and IL-13 from cells of both the innate and the adaptive immune systems, is currently been described from cellular, to molecular and transcriptomics levels and specific targets for pharmaceutical intervention are available or under clinical investigation [11]. It is nowadays unknown whether the Th2-high phenotype of asthma is also characterized by a peculiar underlying structural airway change. In this review we propose point by point how cells of the natural and adaptive immune system orchestrate in synergy the development of the structural hallmarks of airway remodelling. (Table 1).

Some of the known Th2 induced inflammatory effects, as mucous production and ASMC hyperplasia, are overlapping phenomena with remodeling. Whereas other cells and mediators exert different actions either in the context of inflammation or in the structural remodeling. This is the case of eosinophils, neutrophils, mast cells, cytokines, chemokines, and growth factors.

4.1. Eosinophils

Airways eosinophils are the hallmark of asthma inflammation, usually present in increased number in asthmatic airways within different sputum cellular patterns according to identifiable phenotypes. Airway eosinophilia is independent of the atopic status and nowadays a recognized biomarker for the Th2 endotype of severe asthma [12]. Eosinophils can produce many molecules implicated in the remodeling process [13]. Eosinophils are the main source transforming growth factor-beta (TGF- β) in asthma, a cytokine that acts both locally as part of a large latent complex linked to the extracellular matrix or distally after detaching from the complex. TGF- β induces proliferation and regulates fibroblast function as well as controls the production of ECM proteins. Besides, TGF- β has been shown to induce ASM hypertrophy.

The inhibition of the downstream TGF- β signaling in knockout mouse for Smad 3 molecule is associated, after ovalbumin (OVA) challenge, to weaker peribronchial fibrosis, SM layer thickness, and mucus production as well fewer myofibroblasts. [14].

Bioptic analysis after intradermal allergen challenge showed a late-phase reaction characterized by persisting TGF- β 1(+) eosinophils, myofibroblast formation and synthesis of tenascin and procollagen. In the same experiment culture of primary human dermal fibroblasts with

Table 1
Effects of immune cell mediators on airway remodeling features according to mouse and human experiment as reported in the text.

Cell mediator /receptor	SA #	Exp model, Ref	BM ^s	Mucus secretion	ECM synthesis/fibrosis	EMT/ fibroblast	FMT	ASMc Proliferation/ hypertrophy	angiogenesis	Airway inflammatory cells	Lung function	mediators	Remodeling biomarker
Eosinophils													
TGF-β1	+	H _(c) [15]			+ tenascin, procollagen, fibronectin		+	+		↑ Eos, MCs	→ AHR	↑TNF-α, IL13, MMP-2 ↓BNP-4,7	BNP-4 [154]
IL-5	-	M _{KO} (ov)/smad3 [14]	-	-	-		-	+		↓ eos			
	+	M _{TG(ov)} I [17]	+	+	+		+	-		↑ eos	→ AHR	↓ TGF-β	
	-	M _{KO} [16]			-			-		↓ eos		↓ TGF-β	
CCR3	-	H _(Mab) [19,20,21]			- tenascin, lumican, collagen III					↓ eos	↑ → FEV ₁		
Th2 cells	-	M _{Mab(ov)/HDM} [22]		±	- / ±			±		↓ eos			
CD4	-	M _{Mab(ov)} [23]	-		-			-		↓ eos, DC, CD8 +		↓VCAM-1, ICAM-1, CD44	
IL-13	+	H _(c) , M _(c) [24,25]	+	+	+ collagen I		+	+ (co)		↑ eos	↓FEV ₁ *	↑TGF-β1,2 MMP-2, PDGF-A,	Periostin, DDP-4 [138,139,140]
	+	M _{TG(ov)} [28]		+	+ hydroxyproline			±		↑ eos	↑AHR	MMP-12, MMP13, MCP-1	
IL-4	-	M _{KO} (HDM) [29]		-	-			±		↓ eos	↓AHR		
	-	H _(Mab) [30]								↓ eos	↑ FEV ₁		
	+	H _(c) [36]			+ collagen III, procollagen I			+		↓ eos			
	-	M _{KO} (ov) [33]		-	-			±		→ eos	↓AHR		
	+	M _{TG(ov)} [34]		+									
	+	M _{TG(ov)} GATA-3 [35]	+	+	+ procollagen			+		↑ eos		↑IL-4, IL-5, IL-13, eotaxin, TGF-β	
IL-9	-	M _{KO} (ov) [37]		-	- collagen			-		↓ MCs		↓ TGF-β, VEGF, FGF	
IgE	+	H _(c) [43]	+	+	+ collagen I, III, VII, fibronectin			+ (co)		↑ eos, ↓neu	↑AHR	↑ IL-4, IL-5, IL-13, IL-6, IL-8, TNF-α	Galectin-3 [41]
	-	H _(Mab) , M _(Mab) M _{KO} (ov)/gal-3 [44,45,46]	-	-	- collagen III, V, hydroxyproline			-		↓ eos	↓AHR		
Mast cells													
MCs	-	M _{KO} [47]		-	- collagen					↓ eos	↓AHR		
AREG	+	H _(c) [48]		+		+				↑ILC2			
tryptase	+	H _(c) [50]			+ collagen	+		+	+		↑AHR	↑MMP-1, TGF-β	Tryptase [50]
chymase	+	H _(c) [51]			-	+		-	+			↑sCD44, fibronectin, MMP-9	
Activin A	+	H _(c) , M _(c) [53,54]	+					+		↑ eos	↑AHR	↓EGF	
	-	M _(Mab/ov) [55]	-		-							↑TGF-β, IL-25	
Th17												↓TGF-β, IL-4, IL-5, IL-13, IgE	Follistatin [55]
IL-17A	+	H _(c) , M _(ov) [57-58,59,60]		+	+ collagen I, procoll1a2	+		+	+	↑neu	↑AHR	↑IL-8, MPO, TGF-β, IL-9, MCP-1, MMP-9, RANTES, PLA2, VEGF	Wnt5a, I [132], sIL-17A, YKL-40 [159,160,161]
	-	H _(Mab) , M _(Mab) , M _(c-s) [6566]	-	-	- collagen I and III, decorin, actin, biglycan, lumican, fibronectin				-	↓ Neu, ma, →eos	↑ →FEV ₁	↓IL-6, MMP-9, MMP-12, TIMP-1, TGF-β	
IL-22	+	M _(LPS/ATP-DC) [62]	+	+		+		+		↑neu	↑AHR	↓IL-13, IL-33	
	+	H _(c) , M _(ov) [64,74]		+						↑↑			
AECs													
IL-25 (IL-17-E)	+	H _(c) [53],					+			↑ eos	↓ →FEV ₁	↑IL-4, IL-5, IL-13	(continued on next page)

Table 1 (continued)

Cell mediator /receptor	SA #	Exp model, Ref	BM ^s	Mucus secretion	ECM synthesis/fibrosis	EMT/fibroblast	FMT	ASMc Proliferation/hypertrophy	angiogenesis	Airway inflammatory cells	Lung function	mediators	Remodelling biomarker
IL-33	+	H _(C) , M _(C) [76,77,80]	+	-	+ collagen I,III,V, fibronectin	+	+			↑Th2, IL2, Treg, MCs, Ma	↑AHR	↑IL-4, IL-5, IL-13, CTGF, FGF, MMP-1, MMP-9, MMP-12, MMP-13, AREG, eotaxin ↓ (see IL-33)	AREG [49]
TSLP	-	M _{(KO)HMOsT2} [78] M _(KOAb) [79] H _(C) [82] M _{(KOAb)STAT3} [83,84] H _(C) [81]		-	- collagen I, III, V, fibronectin + collagen I - collagen I		+	+		↓MCs, Ma	↓AHR	↑IL-4, IL-13, ↓e-cadherin ↑IL-6, IL-8, GM-CSG	
IL-1α	+	M _(KO) [68,75,107]		+	+ collagen	+	+	+		↑neu →	↑AHR,		
Endothelin-1	+	M _(KO) [70] [73],		-	+ collagen		+	+		↑eos		↑TNF-α, TGF, IL-17 ↑EGFR, FGF-2, CTGF, VEGF, IL-6, IL-8, IL-17 ↓COX, PGE2, ADAM33	
EGF	-	M _(KO) [71]		+	-		+	-				↑alpha(v)beta3 integrin, IL-13, MMP-9 ↓TGF-β, VEGF, MMP-2	s-osteopontin [104].
TGF-β1,2	+	H _(C) , M _(KO) [95,96]		+	+ perlecan	+	+	+	+		± AHR	↓IL-1β, TNF-α	MMP-9 [110]
osteopontin	+	H _(C) , M _(KO) [103,104]		+		+	+	+					
neutrophils	-	M _{(KO)KO} [102]		-		+	+	-					
MMP-9	-	M _{(KO)KOAb} [111]		±	-		+	+					
fibroblasts	+	H _(C) , M _(C) [94]				+	+	+					
FGF-2	+					+	+	+					
CTGF	+	H _(C) , M _(C) [96,97]	+		+ fibronectin, collagen I, tenascin, decorin		+	+	+			↑PDGF, insulin, endothelin-1, TGF-β, ED-A fibronectin, IL-4, IL-13 ↑VEGF	
tumstatin	+	M _(C) [105]	-		-		+	+		↓neu ↑Eos		↑MMP-1, MMP-10 ↑TGF-β, MCP-1, perostin	
MMP-2	+	H _(C) , M _(TG) [112]		+	+ collagen I		+	+					
Cell mediator /receptor	SA #	Exp model	BM ^s	Mucus secretion	ECM synthesis/fibrosis	EMT/fibroblast	FMT	ASMc Proliferation/hypertrophy	angiogenesis	Airway inflammatory cells	Lung function	mediators	Remodelling biomarker
ASMc													
endostatin	+	M _(KO) [89]		±	- collagen IV			+	-	↑eos	↓AHR	↓VEGF, IgE	
ADAM33	+	M _(KO) , M _(TG) [113,114] H _(C) , [91]			+ collagen I,III	+		+	+	→	↓FEV ₁	Fibronectin, Pecam1 →IL-5, IL-13, CCL1 ↑RANTES, eotaxin, GM-CSF	sADAM33 [115]
Fibronectin	+						+	+		↑eos ↓eos → IgE	→FEV ₁	↑GM-CSF, IL-13	
PDGFA	+	H _(C) , [98] H _(KOAb) , M _(KO) , RTKs inhibitors, [133]			+ procollagen	+	+	+					
BDNF	+	M _{(KO)HMOsT2} [100,119], M _{(KO)HMOsT2} , H _(C) , [118].		+	+ collagen-1 and collagen-3			+	+		↑AHR	↑MMP-9, MMP-2	
M2/3 Receptor	+				+ collagen			+	+				
Abl	-	M _{(KO)KO} [125] H _(C) , M _(KO) [120]		-	- collagen, fibronectin			-	-	→ eos		↑TGF-β, LTC4, LTD4, PG, IL-8	
TS2Rs	+	H _(C) , M _(KO) [127]		+		+		+			↑FEV ₁	→IL-13 ↓PGE2, LTD4 MMP-8 MMP9 MMP12, TGF-β	

(continued on next page)

Table 1 (continued)

Cell mediator /receptor	SA #	Exp model, Ref	BM [§]	Mucus secretion	ECM synthesis/fibrosis	EMT/fibroblast	FMT	ASMc Proliferation/hypertrophy	angiogenesis	Airway inflammatory cells	Lung function	mediators	Remodeling biomarker
endothelium													
VEGF	+	M _(ov) [134] M _{MoaAb} [135]	+		+ collagen IV, fibronectin			+		↑eos	↑AHR	↑AREG, active A	

M = mouse ; H = human; (KO) = knock out; (ov): challenged with ovalbumin; (C) = cell cultures or biopsy; HDM challenged with house dust mite; (Mab) = Monoclonal antibody; (LPS/ATP-DC) = allergen pulsed dendritic cells activated with LPS and ATP-(a-stat3) = stat-3 inhibitor; (tki) = tyrosine kinase inhibitor; FEV₁ = expiratory forced volume in the first second; ST2 = receptor for IL33; Ab1 = non receptor tyrosine kinase; INMPP1 = PP1 derivative receptor TrkB agonist-; RTKs = Receptor tyrosine kinases (RTKs) ; gal-3 = galactin 3.
= state of activation ; § = basement membrane thickness ; * = IL13Q110 genetic variant; (co) = muscle contraction.

human eosinophils resulted in a marked transformation in myofibroblast-like phenotype in a time-dependent manner. [15].

The release of TGF-β from eosinophils is enhanced by tumor necrosis factor-α (TNF-α) derived from mast cells that through tryptase also induces fibroblasts proliferation. In turn, fibroblasts promote survival of MCs and eosinophils by releasing secrete stem-cell factor (SCF) and granulocyte-macrophage-colony stimulating factor (GM-CSF).

4.2. IL5 and eosinophils chemokines

IL-5 is the cytokine mainly implicated in eosinophils maturation, survival and migration into the airways. Th2 cells and type 2 innate lymphoid cells (ILC2s) are primarily responsible for the production of high levels of T2 cytokines like IL-5, IL-4, IL-13 and IL-9 in the airways [12] representing the signature for 'type 2 (T2) asthma'.

Murine IL-5 deficient model chronically challenged with an allergen showed a reduction in remodeling signatures like peribronchial fibrosis and smooth muscle thickness and resulted in a decreased presence of TGF-β positive cells in the peribronchial layer. [16]. An opposite effect was found in mice overexpressing IL-5 and eotaxin genes following OVA sensitization and challenge [17].

Anti-IL5 biologics are currently recognized strategies for severe eosinophilic asthma treatment guided by an approach to precision medicine [18]. The efficacy in the appropriate phenotype has been targeted on the demonstration of a significant decrease in asthma exacerbations and the parallel blocking of the IL5-mediated eosinophilic inflammation. Mepolizumab, a humanized IgG monoclonal antibody with a high affinity for IL-5, has been shown to decrease the deposition of tenascin, lumican and collagen III in the RBM of mild atopic asthmatics as well the number of eosinophils and the degree of TGF-β in the bronchoalveolar lavage fluid (BALF) [19]. Lung function was not expected to be positively modified by anti-IL-5 treatments in severe asthma and this effect appears mild when looking at a meta-analysis of nine randomized, placebo-controlled, clinical trials including mepolizumab or reslizumab reporting an absolute difference of FEV1 of 112.93 ml in favor of the anti-IL-5 treatment compared to placebo [20]. However, Benralizumab, which binds the α subunit of the IL-5 receptor, results in a complete depletion of eosinophils in the airway lumen and this can in part explain why in the registrative studies pre-bronchodilator FEV1 improved in the treatment groups [21].

Eosinophils are attracted into the airways through the binding of the chemokines eotaxins, eotaxin-3, monocyte chemotactic protein 3 and 4 (MCP-3, MCP-4) and RANTES to the C-C chemokine receptor 3 (CCR3). By blocking CCR3 with an antagonist, an effect on remodeling in terms of peribronchial fibrosis was observed when mouse models were challenged with OVA neither with chronic house dust mite (HDM), suggesting that persistent rather than repeated short trigger evokes different effects on remodeling. [22]. These data seem to be in favor of a modifying disease progression effect of targeting eosinophils in asthma, nevertheless the link between eosinophilic airway inflammation and remodeling is a matter of debate.

4.3. Th2 lymphocytes

Certain features of airway remodeling, including subepithelial fibrosis, epithelial thickening, mucous cells hyperplasia are significantly reduced by depletion of CD4+ cells. These effects in BALB/c mice after allergen challenge are dependent on the timing of depletion. Actually, when antibodies against CD4+ cells were administrated during chronic exposure to OVA, and not before the challenge, all the aforementioned effects were abolished, although peribronchial inflammation in terms of eosinophilia, dendritic cells (DCs) and CD8+ cells were reduced [23]. T cells accumulate, close to MCs and eosinophils, in the submucosal layer, where they can also adhere to human ASMc through integrins such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and CD44 and can induce ASMc proliferation.

4.4. Th2 cytokines

IL-13 is produced by basophils, DCs, ASMcs, MCs other than Th2 lymphocytes and exerts its effects by binding the α chain of the IL-4 receptor. First, IL-13 is able to induce goblet cells metaplasia and to increase mucus production. Moreover, this cytokine promotes subepithelial collagen deposition by the transformation of airway fibroblasts into myofibroblasts. Actually, IL-13 stimulates collagen type-1 production by the airway fibroblasts cultured from endobronchial biopsies in a matrix metalloproteinase 2 (MMP-2) and transforming growth factor (TGF)- β 1-dependent manner. IL-13R α 2 receptor subunit is considered a decoy receptor that negatively regulates IL-13 signaling, and its relative deficiency in asthmatics compared to healthy controls may explain enhanced airway fibroblasts responsiveness to IL-13. In addition, TGF- β 1 has been shown to augment IL-13 signaling by lowering the expression of IL-13R α 2. The capacity of IL-13 to induce the differentiation of fibroblasts into myofibroblasts is supported by its effect on the expression of alpha-smooth muscle actin (α -SMA) and desmin, two known markers for myofibroblasts [24]. In primary cultured mouse airway fibroblasts, IL-13 time-dependently induces collagen I production mediated through JAK/STAT6 signal pathway and upregulates platelet-derived growth factor-A (PDGF-A) that in turn activates ERK1/2 MAPK and causes collagen production [25]. The genetic variant IL13Q110 of a single-nucleotide in IL-13 gene is associated not only to increase Th2 effector function in asthma but also to a decline in pulmonary function in terms of FEV1. This variant showed a lower affinity with the aforementioned IL-13R α 2 chain [26]. Another IL-13 gene variant, homozygous for AA of rs20541 has been implicated in the effects of high-dose inhaled corticosteroids on reversing decreased pulmonary function, decline in FEV1 and subepithelial layer thickness of biopsy specimens [27].

Transgenic mice overexpressing IL-13 resulted in lung mononuclear cells inflammation, subepithelial fibrosis, mucous cell hyperplasia. All of these effects depend on STAT6 signaling [28]. Again both mucus production and subepithelial collagen deposition, as well AHR, are effects reversible in the model of IL-13 deficient mice repeatedly challenged with HDM. [29]. Moreover Lebrikizumab, a humanized anti IL-13 mAb, improved FEV1 in patients with uncontrolled asthma, as well in a randomized, double-blind, placebo-controlled, phase 2b trial, Tralokinumab, another human interleukin-13 neutralizing monoclonal antibody, induced a significant increase in prebronchodilator FEV1 compared with placebo when administered every 2 weeks but not every four [30]. High periostin levels at baseline (> 50 ng/ml) identified a subgroup of patients with a more pronounced clinical effect albeit globally mild. Periostin is an extracellular matrix protein secreted by airway epithelial cells and lung fibroblasts in response to stimulation by IL-4 and IL-13 and it is implicated in subepithelial fibrosis by binding to other ECMs [31]. Dipeptidyl Peptidase-4 (DDP-4), a serine protease enhanced in airway bronchial epithelial cells of asthmatics, is a profibrotic molecule induced by IL-13. DDP-4 serum levels are potential candidate as biomarkers of IL-13 pathway activation in asthma. The capability of IL-13 to enhance the contractile processes in ASMcs through the up-regulation of Ca²⁺ sensitizing pathways, represents an add-on effect of this cytokine in the contest of airway remodeling [32]. Finally IL-13 influences metalloproteinase expression, indirectly modulating another way to remodeling.

Mouse models have mainly suggested a role for IL-4 in airway remodeling. After chronic allergen exposure, IL-4 knockout mice were protected from developing collagen deposition and mucin production as well as sustained AHR, whereas the ASMcs increase did not change [33]. On the other hand hyperexpression of IL-4 in the lungs was associated in a mouse model to hypertrophy of epithelial cells, from the trachea to the bronchioles due to the accumulation of mucus glycoprotein [34]. Transgenic mice overexpressing GATA3 (gata 3 tg mice), a key regulator of Th2 development, overexpressed pulmonary IL-4 after repeated allergen challenge coupled with increased subepithelial fibrosis

and ASM hyperplasia [35]. Human fibroblasts after segmental allergen challenge increase the synthesis of α -SMA and collagen III by IL-4 release. [36]. Moreover, IL4 acts in an autocrine way inducing fibroblasts to increase procollagen I transcription and TIMP/MMP-2 ratio.

T helper lymphocytes can produce IL-9. This cytokine acts on MCs, inducing their growth, differentiation, expression of proteases and up-regulation of type I high-affinity receptor for IgE (Fc ϵ RI- α). Again the absence of IL-9 in a mouse model is associated to reduced goblet cells hyperplasia, mucus secretion, collagen deposition, ASMcs proliferation, as a consequence of MCs loss of ability to release TGF- β , VEGF and fibroblast growth factor (FGF) [37].

4.5. IgE

IgE is the hallmark of type 1 hypersensitivity. The high-affinity receptor for IgE, i.e. Fc ϵ RI, is constitutively expressed on mast cells and basophils as a tetramer ($\alpha\beta\gamma_2$). Following receptor cross-linking, a cascade of intracellular events drives Lyn-kinase to phosphorylate immunoreceptor tyrosine-based activation motifs on β and γ subunits, leading to Syk activation [38], which has a pivotal role in cell activation and degranulation. Fc ϵ RI is also expressed on monocytes and DCs in a trimeric form ($\alpha\gamma_2$) [39].

Serum IgE closely correlates with the risk of asthma [40]. Previous data in pediatric cohorts showed that higher serum IgE is associated with atopy (increased aeroallergens sensitization), AHR, bronchial wall thickening, and more severe asthma [41]. A significant inverse association was found previously between total serum IgE and FEV1/FVC independently of smoking and asthma status in a longitudinal evaluation in the general population [42]. Therefore IgE might also be associated with airway remodeling genesis. AECs of asthmatic express Fc ϵ RI and loss of epithelial barrier function could help IgE and allergens to penetrate in direct contact with resident tissue forming cells. ASMcs can be modulated by IgE, through both low and high affinity IgE receptor, in its contraction capacity, in the ability of releasing IL-4, IL5, IL13, IL6, IL8, TNF- α and in ECMs deposition, mainly collagen I, III, VII and fibronectin in a ERK (1/2) and MAPK dependent manner [43]. The clinical efficacy of omalizumab, a recombinant humanized IgG1 monoclonal anti-IgE antibody, in severe persistent allergic asthma, has intrigued researchers over the possible add-on effects on remodeling. Omalizumab is able not only to reduce free circulating IgE, but down-regulate the expression of its receptors on effectors cells, antigen-presenting cells, and epithelial cells. Moreover, it reduces sputum and airway eosinophilia, exhaled nitric oxide (FENO) levels, and the bronchial infiltrating IL-4 secreting B cells and T lymphocytes [44]. The contribution of decreasing airway remodeling in omalizumab treated patients is indicated by different experimental findings. In an OVA-sensitized murine model, omalizumab decreases peribronchial collagen III and V, hydroxyproline, and α -SMA. IL-6, IL-8 and TNF- α secretion by IgE, as well as collagen deposition, were abolished by omalizumab in experimental ASMcs cultures. Histopathological observations on bronchial biopsy from severe asthmatics before and after 1 year of omalizumab treatment demonstrated a significant reduction in RBM thickness and in the number of infiltrating eosinophils. A proteomic cluster analysis of these samples resulted in the downregulation of bronchial smooth muscle proteins, among which galectin-3, an ECM protein, resulted in the more reliable marker for airway remodeling [45].

Omalizumab showed an add-on contribution to the persistent reversal of airway obstruction in a consistent number of patients with severe allergic asthma that showed FEV1 normalization already after 1 year of treatment. This effect on lung function was associated with a severe asthma phenotype characterized by higher values of circulating eosinophils, exhaled nitric oxide (FENO), and prevalence of rhinitis and nasal polyps [46].

4.6. Mast cells

MCs are implicated both in the immediate hypersensitivity and the late phase reactions of the allergic and inflammatory response in the respiratory tract. MCs exert their effects by releasing preformed and de-novo synthesized mediators. In MC deficient mice models both FcR γ dependent and FcR γ independent pathways of activation resulted important, at different degree, for the development of chronic asthma features, including goblet cell hyperplasia, increase of mucin genes MUC5AC/MUC5B and collagen levels [47].

A direct interaction between MCs and mucus glands has also been described. Amphiregulin (AREG), a member of the EGF family, is secreted by humans MCs upon allergen stimulation causing upregulation of mucin gene expression in AECs and goblet cell hyperplasia of asthmatics. Moreover, AREG is able to induce the proliferation of fibroblasts [48].

It has been recently proven that interleukin-33 (IL-33) enhanced amphiregulin production by T helper 2 (Th2) cells that instruct eosinophils to secrete osteopontin and facilitate airway fibrosis [49].

Many other mediators derived from MCs are proposed for a role in remodeling pathogenesis. Tryptase has been shown to induce fibroblasts, ASM c , epithelial and endothelial cells proliferation [50]; airway matrix metalloproteinase-1 (MMP-1) is activated by MCs tryptase, induces proliferation of ECMs and is associated to AHR. Chymase degrades basement membrane component by MMP-9, release fibronectin and soluble CD44, reduce EGF-induced ASM c proliferation [51]; activin A, TGF- α , PDGF and TGF- β overall increase ASM c mass [52].

Activin A is a pleiotropic cytokine holding a fundamental role in inflammation and tissue remodeling. The activin beta-A gene is strongly induced in human mast cells.

Activin A in sputum and BALF of asthmatics correlated with reticular basement membrane (RBM) thickness. In airway epithelial cells overexpressing Smad 2, a transforming growth factor-beta signaling molecule, activin A was increased in association to IL-25 as the results of HDM challenged mice and mediated the enhanced airway remodeling and AHR. [53]. The bioactivity of activin can be modulated by follistatin, an antagonist that inhibits asthmatic airway remodeling. [54,55].

MCs are in turn a target for ASM c released molecules. C-X-C motif ligand 10 (CXCL10, also known as Interferon gamma-induced protein 10) is a chemokine acting on its receptors CXCR3 expressed on human MCs that are localized in smooth muscle and here migrated. Fractalkine, constitutively produced by human ASM c , becomes able to attract MCs after pretreatment with neuropeptide vasoactive intestinal peptide (VIP), released upon proinflammatory stimulation. [56]. A cell-cell interaction by adhesion of MCs to ASM cells is critical for their activation and is mediated by tumor suppressor in lung cancer -1 (TSLC-1) acting as a heterophilic adhesion molecule, VCAM-1 and ICAM-1.

4.7. Th17 cells

Th17 are a subset of T helper lymphocytes implicated in the pathogenesis of asthma. Th17 activation depends on IL-1 β , IL-6 and IL-23 produced by DCs in response to pattern recognition receptors (PARR) interaction with their ligands. In humans, the most important cytokines produced by Th17 are IL-17A, IL-17 F and IL-17E (named IL-25), IL-22 and IL-21. Overexpression of IL17 mRNA is found in the airways of murine model of asthma, associated with methacholine challenge AR, as well as soluble IL-17A in BALF of severe asthmatic patients. These findings were replicated in sputum from mild to mild-moderate and severe asthmatics. Particularly in the latter group the increase of IL-17A protein corresponded to neutrophil count, IL-8 and myeloperoxidase levels [57].

Immunoreactivity for IL-17A in bronchial tissue biopsy from moderate to severe asthma appeared increased both in the sub-epithelial and intraepithelial layer. IL-17A outgrowths in parallel with TGF- β and

type-1 collagen [58]. IL-17A activates fibroblasts and epithelial cells to produce neutrophil chemoattractant molecules, including CXCL-8 (IL-8) and results in neutrophilic inflammation that has been associated with disease severity. This kind of inflammation leads to tissue damage and consequent repairing processes [59].

Mucous cells hyperplasia and mucin gene expression is induced by IL-17A in a mouse model of airway inflammation and differentiated human AECs respectively. An essential role of Th17 cells in excessive mucus secretion has been proposed through a mechanism independent from the IL13-STAT6 pathway and is RSV induced.

Th17 has been shown to induce airway smooth muscle proliferation. Actually, ASM c express the receptors for IL-17 and IL-22 (IL-17RA, IL-17RC, IL-22R1) whose ligation induces ASM s proliferation and migration not only in asthmatics but also in healthy controls. In addition, IL-17A increases ASM c contractility contributing to AHR. All the aforementioned effects on ASM c follow the activation of MAPK, RhoA and NF κ B signaling pathways [60]. A study from Kaminska *et al*, when assessing airway remodeling outcomes comparing patients with severe asthma with and without chronic persistent airflow obstruction, reported higher IL-17A levels in sputum in subjects with intermittent reversible obstruction coupled with IL-9, monocyte chemotactic protein 1 (MCP-1), and RANTES [61] thus again relating Th17 to AHR, later onset and shorter disease duration.

The DC phenotype is crucial in orientating a pro-remodeling Th17 response. Intranasal application of allergen-pulsed high dose LPS/ATP-activated DCs in BALB/mice caused a significant increase in IL-17A production and neutrophil infiltration in the airway lumen and Th17 ROR γ t infiltrating cells. Measurable effects on remodeling in terms of goblet cells count, thickness of BM and α -SMA correlated to IL-17A and TGF- β levels. Moreover, freshly isolated lung fibroblasts stimulated with IL17-A, showed an increase of the fibrotic matrix component proCollagen1a2 (coll1a2) and TGF-1 β expression and this effect is significantly enhanced by the combination of IL-17A and the profibrotic factor Wnt5a (wingless-type MMTV integration site family, member 5). Therefore IL-17A may act directly via IL-17R or by TGF-1 β signaling on lung fibroblasts. Wnt5a multiplies the TGF-1 β signal in ASM c and is produced by epithelial cells and macrophages, in a non-neutrophil associated pathway [62].

In a prolonged OVA-challenged mouse model, an exaggerated airway vascular remodeling, by accelerating endothelial progenitor cell (EPC) chemotaxis and pulmonary microvascular endothelial cells formation (PMVECs) was associated with the level of airways infiltrating Th17 cells and IL-17A but not IL-17 F [63].

The role of the other Th17 derived cytokines on airway inflammation and remodeling is quite controversial. IL-22 plays a dual role with pro and anti-inflammatory effects in allergic airway inflammation, and as mentioned before, increases ASM c proliferation via NF κ B [64]. IL-17E, notably none as IL-25, also produced by lung epithelial cells after allergen exposure, is required for initiation of Th2-mediated responses and is detectable in eosinophils infiltrating bronchial submucosa of asthmatic patients. Overall Th17-derived cytokines interact with structural cells of the airway to induce pathophysiological processes that impact ASM functionality.

Neutralizing IL-17 through monoclonal antibodies has failed to induce significant clinical effects in different cohorts of asthmatics. A mild bronchodilator reversibility effect has been proved for brodalumab, an IL-17RA antagonist compared with placebo. OVA-LPS-treated animals treated with anti-IL-17 showed decreased pulmonary inflammation, reduced IL-6 gene expression, oxidative stress, and extracellular matrix remodeling markers, including MMP-9, MMP-12, TIMP-1, TGF- β and fraction of collagen fibers I and III, decorin, actin, biglycan, lumican, and fibronectin [65]. To be mentioned that strategies targeting IL-4 IL-13 or IL-4Ra concomitantly decrease Th2 mediated and eosinophilic inflammation yet may increase Th17 neutrophil inflammation.

STAT3 (signal transducer and activator of transcription 3)

activation promotes the transcription of Th17 associated genes, but it is also involved in Th2 cytokines production. In a mouse model of HDM induced asthma, both Th2 and Th17 cytokines contribute to airway inflammation and remodeling in a STAT3 dependent way. All the effects were completely blocked by systemic administration of STAT3 inhibitor including the increase in goblet cells hyperplasia and epithelial and smooth muscle thickness. [66]

4.8. Neutrophils

Neutrophilia, in the context of asthma, is associated with more severe disease but the non-eosinophilic phenotype of asthma is also common in milder forms. Neutrophilic inflammation is associated with Th17 responses that, as previously shown, contribute to structural changes of remodeling.

A subset of blood neutrophils, expressing CD177, is able to secrete IL-17A in response to fungal allergens. Transgenic expression of IL-8 in bronchial epithelium mimicked in mice severe asthma with a neutrophilic phenotype and progressive airway remodeling. It is interesting to highlight that either isolated HDM or diesel exhaust particles (DEPs) in murine asthma models induce neutrophilia, whereas the co-exposure of HDM and DEPs brings to eosinophilia. One key molecule in the relationship between neutrophils and remodeling is MMP-9, which is released from neutrophils after allergen stimulation in asthmatics. MMP-9 correlate with neutrophils levels when asthma is associated with smoke, bronchiectasis, persistent airway obstruction and reduced airway lumen [67] Severe asthmatics on a high level of inhaled or systemic corticosteroids display high numbers of sputum neutrophils. This is explained by inhibition of the apoptosis of neutrophils, ATP released from dying cells and neutrophil survival induced by LTB₄. This steroid-dependent persistence of neutrophils in the airways of asthmatics can contribute to remodeling [68].

5. Epithelium

The bronchial epithelium functions as a barrier to chemical, physical and immunological stimuli that induce injury and the consequent repairing response to maintain the tissue homeostasis. In bronchial asthma, it is now recognized that in response to microorganisms, respiratory viruses, air pollutants, and allergens, the airway epithelium orchestrates the immune response in the lungs, by supporting the activation, recruitment, and mobilization of immune cells. The most characterized inflammatory response to the previous noxae by AECs involves the release of the cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) able to originate a downstream cascade starting from recruitment and activation of specific immune cells, such as DCs, type-2 innate lymphoid cells (ILC2s), MCs, eosinophils, neutrophils, and basophils, and thus promoting the production of Th2 cytokines, primarily IL-4, IL-5, and IL-13 [69].

Besides in response to injury, epithelium establishes a series of repairing processes that play a key role in driving airway remodeling. Step by step stages of epithelial repair in asthma is described. The first is an immediate response to growth factors as TGF- β and epidermal growth factor (EGF), inducing cell migration, the formation of a temporary barrier, the so-called epithelial-to-mesenchymal transition (EMT), and metalloproteases and collagen synthesis coupled with downregulation of tight junctions. The underline fibroblasts layer responds by differentiating into myofibroblasts. An interim repairing stage follows during which epithelial cells divide to replace lost cells and differentiate into goblet cells meanwhile the transitory matrix remodels. The final stage consists of further differentiation and proliferation and myofibroblast apoptosis [70]. The coordinate action in directing immune responses to environmental stimuli between the epithelium and the beneath fibroblasts sheath is named epithelial mesenchymal-trophic unit (EMTU). [71]

EMT can be demonstrated by decreased expression of epithelial

markers (E-cadherin and MUC5AC) and increased expression of mesenchymal markers (N-cadherin and vimentin) and EMT-associated transcription factors (Snail1 and Zeb1).

5.1. Growth factors

In asthma, these mechanisms of injury and repair are abnormal, as well as epithelial tight junctions integrity. The abnormal epithelial function is evident not only in children and adults with established asthma but also in pre-school wheezers where a new susceptibility gene, CDHR3 (encoding cadherin-related family member 3), highly expressed in airway epithelium, could have a predisposing role. Environmental factors or mechanical stress result in epithelial injury and subsequent release of mediators that contribute to remodeling. In this sense, asthma has been proposed as a disorder that starts in epithelial-mesenchymal interactions.

These cross-talk mechanisms are demonstrated in biopsies or *in vitro* culture models by an increased expression of EGF receptors, the more extensive EMT in response to TGF- β , the marked synthesis of collagen and other extracellular matrix component and the increased myofibroblasts differentiation. TGF- β 2 from epithelial cells have been shown to increase after both allergen challenge and scrape wounding in a time-dependent manner. After OVA challenge EGFR is upregulated by epithelial bronchial cells and its activation triggers both mucous gene expression, collagen deposition, ASM hyperplasia, effects prevented by EGFR tyrosine kinase inhibitor tyrphostin AG1478 and BIBIX1522 [72]. The effect of EGFR by one of its ligands, HB-EGF (heparin), derived from AMSc, seems to be strictly correlated to IL-17 produced by Th17 cells. The same ligand may be released by epithelial cells themselves after mechanical stress and bronchoconstriction. Moreover, the co-stimulation of EGFR by TNF- α , EGF, and TGF amplifies the effect on goblet cells [73]. Johnson *et al* demonstrated that in chronically exposed mice to aeroallergens, epithelial cells downregulate tight junction proteins, increase procollagen I and α -SMA and migrate to the sub-mucosa; the contribution of IL-22 to EMT in primary bronchial epithelial cells has been suggested at least in severe asthmatics [74]. In a late-onset mouse model of asthma, remodeling develops despite absent airway inflammation. In this context, ASMc increase and peribronchial collagen deposition were dependent by the epithelial cytokine endothelin-1 [75]

5.2. Goblet cells

Mucus hypersecretion of mucin MUC5AC and MUC5B is one of the most characterizing features of remodeling in asthma. Goblet cells, derived from Clara cells and ciliated cells upregulate mucin synthesis and increase in size in response to many signaling pathways.

Activation of EGFR is essential for mucus secretion and goblet cell metaplasia. The effects of Th2 cytokines, as IL-13 and IL-9 and proinflammatory molecule as IL-1 β , TNF- α and COX2 have already been described. Is to be mentioned the increasing ciliary beat frequency mediated by the bitter taste receptor 2 (TAS2Rs), whose several other functions are below in the text described.

5.3. Alarmins

Alarmins are molecules produced by airway epithelium and generally released when the cell undergoes death and necrosis. Signal for their production is typically viral infection by toll-like receptor 3 (TLR3). Interleukin 33 (IL33) is an IL-1 like pro-inflammatory epithelial alarmin highly expressed in airway mucosa of severe asthma. It binds to its receptor ST2 inducing the expression of Th2 cytokines, mainly IL-4, IL5 and IL13 from T cells and ILC2 cells. [76]

IL-33 has a critical role in maintaining not only allergic airway disease but also structural changes in an independent way. IL-33, acting through ST2, promotes direct activation of fibroblasts and eotaxin

production [77]. Murine surrogates of IL-33 allergen independent asthma administered per-nasally with IL33 resulted in a significant expression of collagen I, III/V and fibronectin deposition and elevated connective tissue growth factor (CTGF) and fibroblast growth factor 4 (FGFR-4) expression with a degree comparable with OVA allergen dependent mice model. Moreover, IL-33 produced an elevated expression of extracellular MMP-1, MMP-12; MMP-13 and transient MMP-9 and TIMP-1, thus regulating the equilibrium between local fibrinolytic activities and inhibitors. All these effects were attenuated in ST2 gene knockout mice challenged with OVA [78]. This profibrotic effects of IL-33 *in vivo* seem to be indirect as the result of interaction with mediators released from other cells, including ILC2s, macrophages, and MCs. The critical network of feedback between IL-33, ILC2s and IL-13 has been reported in a chronic mice model knockout for T cells and ILCs, exposed to different allergens. Furthermore, IL-33 induced the production of AREG by Treg cells and ILC2s that was essential for lung tissue repair after influenza virus infection. The use of anti-ST2 antibody or anti IL33 in mouse models of allergen-induced airway inflammation attenuated mucus hypersecretion, AHR and airway remodeling. [79] In children with severe therapy-resistant asthma, IL-33 increased lung collagen levels and correlated with subepithelial RBM thickness being unaffected by high dose steroids [80]. To be mentioned that the tight interaction with MCs, dispersed within ASM bundles, directly contribute to AHR by the action of IL-33.

Human fibroblasts release proinflammatory mediators by the action of the alarmin IL-1 α induced by stressed human bronchial AECs. IL-1 α is released in the basolateral space in response to HRV infection or dsDNA and IL-6, IL-8 and GM-CSG from fibroblasts are reduced by the IL-1 receptor antagonist (IL-R α) [81] These data suggest that IL-1 α has not only a key role in mediating EMT but expresses the potential to amplify local innate immunity, for example following viral stimulation. This network may involve IL1- α secreted by the macrophages in response to virus and in the ability of MCs to respond to IL-1 α by Th2 cytokine production.

TSLP is secreted in high amounts by AECs in response to epithelial disruption by allergens, viruses, diesel extract particles, microbes, favored by the synergistic activity of IL-4 and IL-13 with the loss of e-cadherin. TSLP binds to its receptor (IL-7 α/γ -chain) expressed in DCs and ILC2s leading to the release of Th2 cytokines, including AREG [82]. TSLP *per se* further disrupts epithelial cell barrier during chronic HDM exposure by activation of activator transcription 5 (STAT5). Bronchial biopsy specimens, collected from patients with asthma, showed epithelial cells overexpressing TSLP, α -SMA and collagen I and TSLP was able to regulate the expression of α -SMA and collagen I by STAT3 activity [83]. Moreover, in asthmatics rats TSLP signaling blockade attenuates airway remodeling in terms of α -SMA and Collagen I mRNA. In another mouse model of asthma sensitization with microRNA-19b reversed remodeling by inhibiting STAT3 signaling through TSLP down-regulation [84]

6. Epithelial-mesenchymal trophic unit

6.1. ECM

The extracellular matrix (ECM) supports structural stability to the airway wall being composed of a network of collagenous and non-collagenous ECM proteins surrounding cells in the airways. The most relevant components of ECM are collagens I, III and V, fibronectin, tenascin, hyaluronan, versican, perlecan and the family of the MMP and their tissue inhibitors (TIMP).

ECM is a dynamic structure in which physiologically turnover and tissue repair are activated. Parts of ECM are constantly replaced by new synthesis, in the case of collagen 15–20% daily to be estimated.

These mechanisms can contribute pathologically to tissue destruction or, as in the case of airway diseases, to remodeling. In asthmatic airway enhanced deposition of RBM determines and increases thickness

with mean values of 9 μ m compared to 5–6 μ m of nonasthmatics. [85]. There is a predominance of accumulation of thinner reticulin fibers rather than thicker interstitial fibers as in other lung diseases. ECM alterations in asthma are visible in the airways, around smooth muscles and vessels and consist in an increased presence of fibronectin, collagen I, III and V, tenascin, hyaluronan, versican, laminin α 2/ β 2, biglycan, MMP-9 and MMP12 and a parallel reduction in PGs that seems to be protective [86]. In severe asthma increased deposition of collagen I and III goes aside increased ASM mass, as well collagen, versican and decorin are associated with uncontrolled clinical asthma. Asthmatic cells produce more perlecan and collagen I but less collagen IV and laminin α 1. In addition, ECM is bioactive and can influence cell proliferation, migration, differentiation and survival. ECM component influence ASM proliferation (fibronectin), survival (laminin) and release of inflammatory mediators (collagen I) that in turn modulate the surrounding ECM.

The main sources of ECM components are fibroblasts and myofibroblasts. They secrete MMPs and their inhibitors, integrate signals from the ECM interacting by integrins signals. In response to injury fibroblasts differentiate into myofibroblasts specialized in ECM production.

6.2. Collagens and other ECM proteins

Collagens are often thought to be inert components yet they participate in the dynamic changes of ECM and may influence inflammatory responses. Collagen I is the major component of ECM that is increased in asthmatics. It exists in microfibrils formed by five monomers assembled from the procollagen derived from polymerization α 1 and α 2 chains.

Collagen I is produced by ASM and fibroblasts, induces ASM proliferation, migration and resistance to the inhibitory effects of glucocorticoids. Collagen I controls the expression and activation of MMP-2 in ASM, inhibits TIMP2, and, in the presence of thrombin, enhances MMP14. In the presence of collagen I, ASM asthmatic cell produces more eotaxin, RANTES, and GM-CSF [87]. Collagen IV is composed of three α -chains whose degradation by MMP, produces endogenous angiogenesis inhibitors, named arrestin, canstatin and tumstatin. The decrease of collagen IV in asthmatics airways and ASM indicates suppressed levels of endogenous angiogenesis inhibitors [88]. Another collagen-derived angiogenic inhibitor is endostatin, derived from degradation of α 1 collagen XVIII chain and acting via inhibition of VEGF signal. In OVA-sensitized mice, administration of endostatin inhibits AHR, allergic and lung inflammation and IgE synthesis [89]. It has also been proposed by some authors that degraded lung collagen function as IL-8 (CXCL8) is chemotactic for neutrophils [90].

Fibronectin is a glycoprotein that polymerizes in the ECM, process that in asthma is inappropriate and exaggerated. Produced by AMSc, fibronectin enhances their proliferation and production of RANTES, GMCSF and eotaxin. Fibronectin is also the component of the matrix that binds chemokines allowing the gradient for cells migration. [91]

Hyaluronan/luronan is a large linear glycosaminoglycan whose receptor, CD44, is constitutively expressed on ASM and bronchial tissue and mediates ASM DNA synthesis by activated T lymphocytes. Hyaluronan interacts also with eosinophils by enhancing their survival and TGF- β production [92].

6.3. Fibroblasts and FMT

Fibroblasts are elongated spindle-like shaped cells dispersed in the connective tissue and attached to the structural network of ECM. Fibroblasts have the capacity of migrating to the injured sites attracted by chemotactic factors including alternative spliced fibronectin (ED-A fibronectin), PDGF and TGF- β secreted by epithelial, inflammatory and mesenchymal cells. Within the site of injury, fibroblasts proliferate and undergo the fibroblast-to-myofibroblast transition (FTM). First

fibroblasts acquire a protomyofibroblast phenotype that exerts transitional forces in the ECM, by expressing β and γ actin and N-cadherin. Subsequently, they differentiate into myofibroblasts under the action of ED-A fibronectin, PDGF and TGF- β and by the effect of mechanical tension [93].

6.4. Profibrotic factors

FGF, a family of 23 homologs, is known to be involved in lung organogenesis and tissue homeostasis and repair. FGF-2 is bound within the ECM to PGs such as syndecans, glypicans, agrin, and perlecan, protecting ECM from proteolysis. Heparinases or other degrading enzymes induce FGF-2 release thus being available for target cells. FGF-2 induces either *per se* fibroblasts, myofibroblasts and ASMC proliferation or it enhances the effects of other ligands such as PDGF, insulin, lysophosphatidic acid, and endothelin-1, TGF- β , IL-4, and IL-13 [94].

TGF- β is able to trigger FTM in asthmatics both *in vivo* and *in vitro* [95]. This cytokine contributes to fibrosis by enhancing the secretion of FGF-2, CTGF, and VEGF from fibroblasts, myofibroblasts, and ASMC.

TGF- β in a potent fibrogenic cytokine produced in an inactive form by many cell types, including eosinophils, epithelial cells, ASMs, macrophages. Its activation from the ECM needs the interaction with different molecules including plasmin, integrins, LPA and thrombin A. The fine mechanisms are described elsewhere in this review [96]. TGF- β is higher in BALF of asthmatics and its gene and staining are hyper expressed in biopsy form bronchial tissue of asthmatics. TGF- β plays a central role in controlling airway remodeling by inducing fibroblast proliferation, ASMC growth and survival, apoptosis of epithelial cells, rise in the production of ECM proteins from fibroblasts and that of the enzymes, namely MMP and TIMP, that regulate their deposition. Pro angiogenic effects are also reported, as well the capacity, elsewhere described, of inducing the release of other fibrogenic factors as FGF [95].

Connective tissue growth factor (CTGF), produced by fibroblasts, epithelial cells, endothelial cells, and ASMC participates in TGF- β mediated fibrosis enhancing fibronectin, collagen I and VEGF production, mainly by ASMC. The autocrine loop is completed in asthmatics ASMC by the enhancing CTGF production in response to TGF- β . CTGF is a sort of bridge between structural proteins and effector molecules such as VEGF. MMP-2 cleaves CTGF releasing VEGF that can reach its receptors [97,96]. Finally CTGF promotes cell adhesion and migration.

PDGF increases lung fibroblasts proliferation, their procollagen I synthesis and favors FMT. These effects are significantly observed in severe asthmatics. As before mentioned PDGF activates eosinophils and induces GM-CSF from ASMC [98].

Factors like NGF, b-PGF, and IGF-1, are in addition to being mentioned as fibroblasts activators [99]. Among neurotrophins NGF is increased in serum and BALF of allergic asthma and is expressed by epithelial, ASMC and inflammatory cells, being enhanced by interferon- γ and IL-1 β . Whereas NGF induce ASMC proliferation, neurotrophin-3 (NT-3) and BDNF are involved in ASMC migration. In any case all the neurotrophins increased MMP-9, but not MMP-2, secretion by ASMC [100]. The activity of IL-13 signaling in fibroblast TGF- β mediated profibrotic effects have already been described [101]. To be stressed that the effect on FMT has linked to the downregulation of cyclooxygenase (COX) gene expression, reduction in PGE2 and activation of the c-Jun NH2-terminal kinase-dependent pathway. Other cytokines may directly or indirectly induce FTM: IL5, IL-6, IL-1, IL-17A, IL-25, IL33, TNF- α and oncostatin M.

Osteopontin (OPN) is increased in the subepithelial layer of severe asthma and, at least in mice, induces FTM. OPN is originally described as a structural component of the extracellular matrix, owning the ability to bind to proteins and most types of collagen. Allergen-challenge model of airway remodeling with OPN KO mice showed that OPN deficiency protects against remodeling and AHR, effects reversed by Administration of rOPN to KO mice, increasing the expression of IL-13

and MMP-9 [102,103]. Significantly increased serum OPN levels were observed in both mild and severe asthmatics, that decreased during exacerbations but were insensitive to corticosteroid treatments. Quantification of lung OPN expression revealed both bronchial epithelial and subepithelial positive infiltrating inflammatory cells, correlating directly to RBM thickness and inversely with FEV1 [104].

Eotaxins, known chemokines for eosinophils, exert bronchial fibroblast activity, proliferation and migration by mechanism MMP-2 mediated. Periostin, a well-established marker of Th2 inflammation in asthma, is a profibrotic factor. Its implication as a proposed biomarker in airway remodeling is discussed later in the text [31].

Tenascin is a myofibroblast marker overexpressed in asthma, and when lacking in animal models, attenuates eosinophilic inflammation. Fibulin-1 is a glycoprotein stabilizing ECM proteins.

Tumstatin is a collagen IV derived matrikine absent in asthmatic airways that in animal models can reverse airway inflammation and remodeling. It is expressed by fibroblasts, AECs, and endothelial cells and suppresses neutrophil chemotaxis when reintroduced to ASMC matrices of asthmatics. Tumstatin induces also an ECM with anti-angiogenic properties, working in synergy with MMP-1 and MMP-10 [105]. Fibroblasts from asthma patients have *per se* an enhanced intrinsic potential to respond to TGF- β and differentiate into myofibroblasts. This potential is connected to the upregulation of α -SMA and connexin (Cx) 43, levels of TGF- β RI receptors, increased rearrangement and deposition of ECM components, increased secretion of growth factors and cytokines (CTGF, IL-6, IL-8, IL-11, IL-17) and TIMP/MMP ratio. The differential response of asthmatic fibroblasts to TGF- β is mediated to the canonical TGF- β /Smad signaling pathway in which Cx43 competes positively with smad2 for binding to microtubules [99].

6.5. Myofibroblasts

Myofibroblasts are bigger, more elongated cells compared to fibroblasts, and have a larger mean surface area. They show an increased expression of components associated with the contractile apparatus, the most important being α -SMA, transgelin stress fibers, non-muscle actin, OB-cadherin, heavy chain myosin, calponin-1, and OPN. In asthma, the persistence of the wound healing process is accompanied by inhibition of myofibroblast apoptosis. Myofibroblasts are interconnected via gap-junction and express integrins, vinculin, paxillin, talin and tensin.

Myofibroblasts contribute in the secretion of ECM components, such as collagens I, III, V, fibronectin, tenascin and proteoglycans, are a source of MMP and TIMP as well growth factors, cytokine and chemokines as GM-CSF, IL-1 IL-6 IL-8, stem cell factor (SCF), TGF- β , VEGF, promoting *in primis* ASMC migration, hyperplasia and hypertrophy [99,82].

Apart from recruitment and differentiation from resident cells, in the fibrotic process are also involved mesenchymal progenitor cells such as fibrocytes, mesenchymal stromal cells (MSCs) and pericytes. The finding of fibroblasts in BALF supports a diverse way from circulating progenitors. Fibrocytes are immune active cells able to present antigens to inflammatory cells by HLA-DR. They are CD34, CD45 CXCR4 positive cells. In particular, the expression of chemokine receptors evokes the capacity of being recruited into the wounded tissue and in asthma, levels of recruited CD34 + /CD45 + / α -SMA fibrocytes correlated to lamina reticularis thickness [106]. In allergen-induced asthma patients bronchial biopsies showed the presence of CD34 + /collagen I + fibrocytes that upon TGF- β and endothelin-1 stimulus acquire a myofibroblast phenotype [107].

The last mechanism contributing to the fibroblast pool in remodeling is, as previously described, EMT. MMPs and TIMPs promote proteolytic digestion of the BM, epithelial cells lose their adhesion and tight junctions, and they convert into mesenchymal cells by the action of TGF- β , insulin growth factor II or FGF-2 [93].

6.6. Metalloproteinases

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade ECM, being involved in extracellular matrix turnover and tissue repair. In addition, they take part in inflammatory response, mucus secretion, angiogenesis and fibrosis. Inflammatory cytokines and pro-fibrotic factors activate the MMP gene family to MMP mRNA transcription and pro-protein MMP synthesis. Pro-MMPs are activated by a cascade of proteolytic activation to catalytic active MMPs. In this form, MMPs can induce different effects: fibrosis and vascular apoptosis/proliferation in the small airway, neutrophilic and macrophagic inflammation, ECM degradation and mucin production. Tissue inhibitors of metalloproteinases (TIMP) regulate MMPs activity both at a post-translational level and in their active form [108]. There are 4 types of TIMPs (1–4) that bind to high affinity to the catalytic site of MMPs. At least 25 MMP family members have been described and grouped into four main categories, collagenases, stromelysins, gelatinases and metalloelastases [109]. MMP-9, named gelatinase B, is produced by macrophages and neutrophils and mildly by epithelial cells, MCs, fibroblasts, and myocytes. It exerts gelatinolytic, elastolytic and collagenolytic activity and modulates the activity of proteinases and their inhibitors or cytokines.

In patients with asthma exacerbation, MMP-9 has been found in blood, sputum and BALF as well elevated MMP-9/TIMP-1 ratio. On the other hand MMP-9 is not associated with atopy [110]. A role in remodeling has suggested by murine MMP-9 deficient asthma models in which fibrosis, by not ASM thickness, mucus secretion and AHR does not develop. Synthetic MMP-9 inhibitors has some beneficial effect in murine models of asthma [111].

Another metalloproteinase involved in asthma is MMP-2, an endopeptidase produced by fibroblasts, Myofibroblasts, AECs and inflammatory cells.

It has been reported that eosinophils indirectly induce FMT by secretion of MMP-2. In detail, eosinophils stimulate secretion of MMP-2 by fibroblasts to activate latent TGF- β 1. Active TGF- β 1 induce FMT as demonstrated by increased α -SMA expression. Myofibroblasts in turn release TGF- β 1 and MMP-2 that break ECM proteins. In addition, MMP-2 activates chemokines and growth factors by proteolysis promoting migration of fibroblasts and eosinophils. Transgenic mice who hyper expressed MMP-2 and received intra-tracheal eosinophils, developed peribronchial inflammation, fibrosis and mucin cells hyperplasia coupled with TGF-1 β , MCP-1, periostin, and α -SMA expression [112]. One additional mechanism suggested is prolonged eosinophils survival by MMP-2 apoptosis inhibition.

6.7. ADAM33

ADAM33 is an asthma susceptibility gene encoding for a multi-functional membrane-anchored glycoprotein including metalloprotease (MP) and disintegrin domains. ADAM33 gene presence is associated with asthma and AR and polymorphisms to a rapid decline in lung function of asthmatics. The ADAM33 protein is selectively expressed in smooth muscle bundles of airways and fibroblasts and its activation induces release of growth factors and upregulation of cell surface receptors.

Increased ADAM33 expression was observed in ASMCs from the OVA-sensitized rats and positively correlated with cell stiffness, traction force, and expression of vinculin and F-actin, suggesting that ADAM33 is a mediator of ASMC dysfunction in asthma.

Shedding of a 55-kD soluble form (sADAM33), including the MP domain, is inversely associated in asthmatic BAL to lung function. sADAM33 may function, as demonstrated in vitro and in vivo assays, as a proangiogenic factor and its effects are enhanced by TGF- β 2. [113]. The soluble form of ADAM33 is also expressed in human airways epithelium acting in the epithelial-mesenchymal differentiation. This phenomenon is associated with the regulation of ADAM33 expression

by TGF β 1. It has been proposed that sADAM33 protein promotes airway remodeling without inflammation starting in early life, determining the susceptibility of the airway tissue to environmental allergens to promote allergic airway inflammation and BHR. In a doxycycline-inducible (Dox-inducible) human sADAM33 transgenic mouse model, mRNA of α -Sma, collagen 1 and 3 (Col1a1 and

Col3a1), fibronectin, and platelet/endothelial cell adhesion molecule 1 (Pecan1; also known as CD31) resulted increased in whole lungs of adult mice as well fetal lungs, while the inflammatory markers, CCL1, IL-5, IL-13, CXCL1, and goblet cell marker, Muc5ac, were not affected. Early-life induction of sADAM33 by Th2 cytokines enhances allergen-induced asthma-like changes potential for early-life interactions between sADAM33 and Th2-type inflammation in response to low concentrations of allergen. [114,96] All together these data support the possibility that ADAM33 may play a role in airway remodeling [115].

Some authors reported the contribution for a prominent role of ADAM33 in asthma pathogenesis as suggested by the upregulation of ADAM33 mRNA expression after allergen-induced chronic airway inflammation due to the activation of C-type lectin receptors (CLRs), Toll-like receptors (TLRs), and aryl hydrocarbon receptor (AhR) on DCs and by epigenetic changes of ADAM33 causing aberrant expression of ADAM33 in epithelial, fibroblasts, and airway smooth muscle cells.

6.8. Mechanical forces

Many studies have reported an effect of mechanical forces in airway remodeling. Among the described effect, mechanical stress increases ECM protein and PG synthesis, MMP-2 and MMP-9 and IL-6 in asthma fibroblasts. The abnormal deposition of ECM matrix protein causes increased matrix stiffness that after injury induces fibroblasts to change and myofibroblasts development, that are able, through a feedback loop, to further promote ECM remodeling and stiffness. [116]

7. Airway smooth muscle cells

ASMCs are active participants in airway physiopathological processes in asthma and transcriptional profiling analysis of ASM of asthmatics showed marked activation of cell proliferation and growth, production and modulation of extracellular components, vascularization, inflammatory response and muscle tissue-specific response implicated in contractility. Overall these actions contribute to airway remodeling.

7.1. ASMC structure

The primary role of ASM is the regulation of airway tone by constriction and dilatation in response to local or systemic factors. In asthma, the increase in airway tone is paralleled by that of ASM mass, both in the number of cells (hyperplasia) and in the size (hypertrophy) and thus constituting the main determinants of airway narrowing and AHR. The involving of both large and small airways in this alteration has been described. An increase in AMS size by 25–55% in asthmatics and 50–200% in fatal asthma have been reported. Early abnormalities are reported in severe wheezers pre-school children who develop asthma.

The increase of bundle thickness but not their orientation represents the main contributor. Moreover, the heterogeneity of ASM area and wall area in asthma increases resistance and elastance. Alteration in ASM contractility in terms of excessive shortening in the most likely responsible of AHR. [10]

7.2. ASM hypertrophy and proliferation

The Increase of ASM mass is a key feature of remodeling associated with the severity of asthma, decreased lung function and enhanced contractility.

Among stimuli inducing ASM hypertrophy mechanical stretch is

very important and acts through microRNA, glycogen synthetase kinase 3b and Akt pathways. TGF- β and endothelin-1 are also hypertrophy inducing factors coupling rapamycin activation.

ASM proliferation in the contest of airway remodeling is controlled by different mechanisms gathered in upregulation of pro-proliferative pathways, inhibition of anti-proliferative or apoptotic pathways or greater proliferation per se. [117]

P38 and p42/44 MAP kinases, PI3/Akt, NF- κ B and TGF- β associated kinases are all enhancing proliferative pathways for ASM, whereas CCAAT/enhancer-binding protein- α with its antiproliferative action is downregulated.

TNF- α , IL-4, IL-5, IL-13, TGF- β , TSLP, and Th17 family cytokines are among the well-characterized proinflammatory mediators inducing ASM hyperplasia. Bronchoconstrictor stimuli able to trigger ASM proliferation are M2 muscarinic receptor agonists, that enhance TGF- β effects, cysteinyl leukotrienes LTC₄ and LTD₄ and prostaglandins. [118]

Other locally produced factors have the ability to modulate ASM proliferation beyond inflammation: EGF produced by epithelial cells VEGF, and the neurotrophin BDNF. Smooth muscle-specific deletion of BDNF reduced AHR and blunted airway fibrosis but did not significantly alter airway inflammation, effects reversed by restoring TrkB signaling [119]. The nonreceptor tyrosine kinase Abl, was upregulated in airway tissues of the animal model of asthma and in airway smooth muscle cells of patients with severe asthma and was associated to smooth muscle contraction and cell proliferation; interestingly IL-13 and CCL2 levels were not concomitantly affected [120]. OPN, upregulated in epithelial cells and macrophages in the lungs, not only induce in mouse models FMT but, when administered to OPN-knockout mice, was able to reverse AHR, collagen and mucus production, gob-5 mRNA expression, submucosal cell area infiltration, and proliferation [102]. Caveolin-1, peroxisome proliferator-activated receptor- γ ligands (PPAR) and vitamin D have been proposed as anti-proliferative molecules. A novel regulatory pathway involving Semaphorin 3A has been shown to induce ASM growth inhibition by binding its receptor neuropilin 1 (Nrp1) [121].

Whether blunted apoptosis of ASMC is also implicated in remodeling it is a matter of doubt. Data from a culture of ASMC report reductive apoptosis induced by Th-17 cytokines, IL-8, eotaxin and MIP-1 α , TRPV1 agonists and laminin. Furthermore, the possibility of ASM autophagy as an adaptive response to survival is not to be ruled out and some data on a role for excessive ROS, accompanying airway inflammation, in this contest, has been suggested.

7.3. ASM and ECM

A strong relationship between ASM and matrix is another determinant of airway remodeling. ASMC are involved in generation of ECM proteins determining different profiles according to the inflammatory milieu that influences ASMs. In turn, ECM proteins can regulate ASMC signals. ASM participates actively in ECM synthesis in response to growth factors and cytokines. [117]

IL-1 β and TNF- α enhance ASM production of MMP-9 and MMP12 that degrade matrix components favoring cell migration and tissue remodeling. TGF- β induce perlecan deposition by ASMC in response to the modulating action of Non-Canonical Wnt5a and β -catenin and rhinovirus. On the other way other mechanisms blunt ECM production by ASMC such as the binding of decorin to TGF- β .

ASMC generate themselves factors that have autocrine/paracrine effects on remodeling, including IL-1 β , IL-5, IL-6, IL-8, IL-17, PDGF, and TGF- β [122].

ASM stimulated by IL-1 β and TNF- α , and regulated by TGF- β , secrete IL-6 that induce ASM hyperplasia; TNF- α enhances also INF- γ secretion by ASMC. ASMC are source and target of TSLP, thus regulating the cytokine network orchestrated by dendritic cells. Emerging evidences support ASM as able to synthesize chemokines such as RANTES,

eotaxin MCP-1 and CXCL10 recruiting inflammatory cells. The autocrine effects on cell proliferation and contractility are further mediated by growth factors like VEGF and BDNF.

7.4. ASM contraction and remodeling

The contraction of ASMC exerts on mechanical properties of the airways chronic effects that go beyond the transient bronchoconstriction, and that can be defined mechanical remodeling. Stiffness is the first determinant of this phenomenon and is associated with the physiological consequences of active contraction short term changes. Canonical activation of the M3 muscarinic receptor cascade induce myosin polymerization, actin polymerization and passive stiffness that ultimately facilitate and augment force production by actomyosin cross-bridges. In addition, cytoskeletal proteins by anchoring integrins and focal adhesion complexes contribute to ASMC stiffness. In asthma sustained levels of contractile mediators, reduced degradation of acetylcholine and reduced endogenous nitric oxide induce a state of increased stiffness in ASMC. The consequence is an alteration in the deep inspiration (DI) reverse bronchoconstriction effect in response to strain, losing the protective and beneficial effect of lung inflation on airway caliber. A second factor that links stiffness and mechanical remodeling is airway narrowing due to a reduction in ASM length with progressive adaption to a shortened length and increased shortening capacity. Both the aforementioned mechanisms, lack of DI response and length adaptation are regulated by increased passive stiffness of ASMC and by force adaptation of cytoskeleton components. [123]

Mechanotransduction consists of transmitting from ECM to ASMC the response to mechanical stimuli (strain) determining the cellular alteration that promotes chronic structural changes over time. Among the mechanisms involved are to be mentioned calcium influx into the cells via stretch-activated channels and caveolae regulation, activation of RhoA/ROCK pathway by a cyclic stretch that stimulates ASM contraction and proliferation, and cell alignment by cytoskeletal remodeling. [124]

Apart from the response to strain, contraction-linked pathways lead to progression in structural remodeling. Muscarinic signaling is able to induce mitogen ASM proliferation, IL-8 production, whereas Metacholine induced bronchoconstriction increases sub-epithelial collagen deposition and mucous gland hyperplasia. Muscarinic receptor-deficient mice (M3 R-/-), exposed to ovalbumin, showed reduced remodeling compared to wild type mice, but inflammation was not reversed, thus sustaining a mechanical way for remodeling independent from inflammation [125]

TGF- β , apart from its known effects on epithelial, mesenchymal and inflammatory cells, can increase expression of contractile proteins like myosin and calponin thus contributing to bronchoconstriction induced remodeling by MCh. TGF- β is secreted by cells in an inactive form called small latent complex (SLC), complexed with a latency-associated peptide (LAP) and binds to proteins of the ECM named TGF- β binding proteins (LTBP). TGF- β can further be activated by different mechanisms. Extracellular portion of integrins interact with both ECM proteins and latent TGF- β complexes, whereas intracellularly integrins interact with actin cytoskeleton by the adaptor protein talin. TGF- β becomes biological active when ASM is contracted, integrins undergo a conformational change that induce detachment of active TGF- β from LAP. Furthermore, stimulation with MCh is also able to activate TGF- β via avb5 integrin. [126] Other cell types, like epithelial and mesenchymal cells are able to activate TGF- β by interaction of integrins and agonists including, LPA and thrombin. A confirm comes from inhibition of avb5 integrin resulting in reduced remodeling in models of allergic asthma. Altogether this data show that uncontrolled bronchoconstriction in asthma leads to activation of TGF- β and promote airway remodeling.

7.4.1. G-protein-coupled receptors (GPCRs)

Contraction of ASMC is strictly linked to mechanisms of ionized

calcium $[Ca^{++}]$ regulation. Activation of G-protein-coupled receptors (GPCRs) mediate bronchoconstriction. Acetylcholine, cyste leukotrienes, histamine, and endothelin are agonists of Gq-coupled receptors and stimulate the production of inositol trisphosphate (IP3) that leads to the sarcoplasmic reticulum (SR) Ca^{++} release. Other GPCR, as Gs-b2-adrenergic receptor (b2AR), induce bronchodilation when agonized. GPCRs alone or in synergy with receptor tyrosine kinase, exert on ASM other effects involving proliferation and growth, secretion of growth factors and release of inflammatory mediators. [117]

7.4.2. Bitter taste receptors

Bitter taste receptors (BTRs) belong to GPCRs and activated by bitter tastants such as chloroquine and saccharine induce bronchodilation. However, they are unexpectedly associated with the elevation of intracellular $[Ca^{++}]$. Bitter taste receptors are expressed in murine ASMs and when activated cause muscle relaxation. They are upregulated in the transcriptomic profile from peripheral blood of severe asthmatic children. Among the mechanisms of action involved, inhibition of bronchoconstriction induced by PGE2, thromboxane, LTD4 and histamine, or membrane hyperpolarization via Ca^{2+} activated K channels (BKca) or I -type channels have been proposed. The most studied BTRs is TAS2R. Its bronchodilator effects seem independent on that of β 2 adrenoceptors. TAR2R agonists limit the proliferation of ASM cells in vitro by inhibiting a plethora of downstream signals in the cell cycle progression. In addition, they promote mitochondrial fragmentation and autophagy restricting cellular proliferation. Treatment of HDM and ovalbumin asthma model with chloroquine and quinine, TAR2R agonists, reversed different aspects of remodeling: expression of calponin, α -SMA and myosin heavy chain was downregulated as well as levels of MMP-8, pro-MMP9 and MMP12, TGF- β and pSmad2. [127]

7.4.3. Other GPCR

Proton-sensing GPCRs (acid-sensing ion channels ASICs) respond to extracellular pH modulating airway reactivity. One of these receptors, OGR1 has been characterized in human ASM contributing to relaxation by modulating the calcium entry in the SR enhancing the activity of Ca^{2+} sensing protein stromal interacting molecule (STIM1) and the plasmic membrane influx channel Orai 1. The implication in asthma exacerbations by acid fog environmental exposure or gastroesophageal reflux are evident. [111] E-prostanoid (EP) receptors are agonized by prostaglandin E2 (PGE2). ASM proliferation is in vitro suppressed by autocrine and exogenous PGE2 after interaction with EP2 and EP4 but not EP3 receptors. [128] Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter able to engage GABA-B receptors expressed in ASM resulting in Gi-coupled cross-activation of Gq-coupled receptors leading to contraction. Baclofen, a GABA-B receptor agonist, worsens AHR in asthmatics. Inhaled dopamine induces bronchodilation in asthma. This effect appears to be mediated by the D2-like receptor Gi-coupled that promotes the bronchodilator action of Gq-coupled b2-adrenoceptors [129,130]. G-protein coupled receptor kinase (GRKs), β arrestins and regulators of G-protein signaling (RGS) may play a role in inhibiting airway contractility, modulating receptors desensitization and remodeling.

Wnt proteins bind the extracellular portion of GPCRs coupled with co-receptors (LPR or RyK) that constitute either the canonical β -catenin dependent pathway or the noncanonical Wnt/Ca system both promoting contractility. Wnt5 is markedly induced in response to TGF- β in ASM, activate both pathways ultimately determining altered airway tone and remodeling. As previously reported Wnt5A enhances ECM production by ASM [131] and has been proposed as a marker of IL-13 and IL-4 treatment groups [132]

7.4.4. Non-GPCR mechanisms of ASM contraction

Receptor tyrosine kinases (RTKs) have a central role in signaling networks involving different cells types in asthma remodeling. The effect of EGFR on ASM and its inhibition has been already pointed out.

As mentioned above PDGF is involved in collagen synthesis by fibroblasts. Activation of PDGFR also induce ASM proliferation in vivo and in vitro, yet mildly mitigated by RTKs inhibitors. The clinical efficacy of masitinib has been dramatically depressed by significant adverse events. Nintedanib, an inhibitor of multiple RTKs and approved in the treatment of unusual idiopathic pulmonary fibrosis (UIP), in a murine model of OVA-induced asthma significantly improved inflammatory and remodeling markers [133].

Receptor tyrosine kinase family of neurotrophic receptors, named tropomyosin-related kinase Trks, are expressed in ASM and upon triggering by the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) enhance contractility. Actually, NGF and BDNF are hyper expressed in asthma and can be released by airway nerves, epithelial cells and ASM itself in response to irritants, cigarette smoke, allergens and infectious agents. BDNF can contribute to ASM proliferation.

8. Vascular remodeling

Vascular remodeling is a characteristic feature of airway remodeling and is due to both angiogenesis and vasculogenesis. In angiogenesis mature pulmonary microvascular endothelial cells (PMVEs) detach from the basement membrane, migrating and proliferating in new vessels. In vasculogenesis, the sources of endothelial cells are bone marrow-derived endothelial progenitor cells (EPCs) that migrate and differentiate in the new vessels.

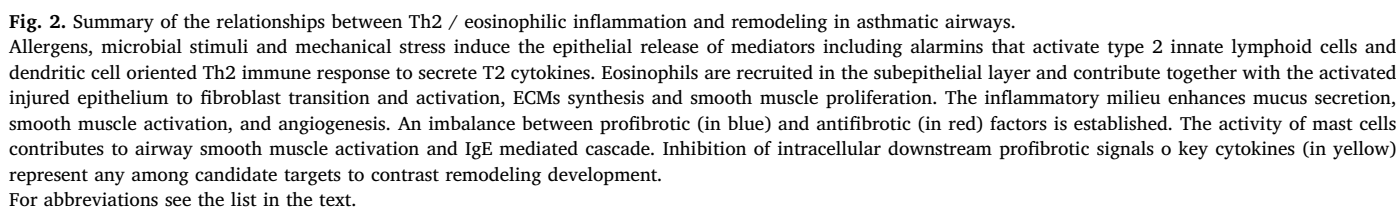
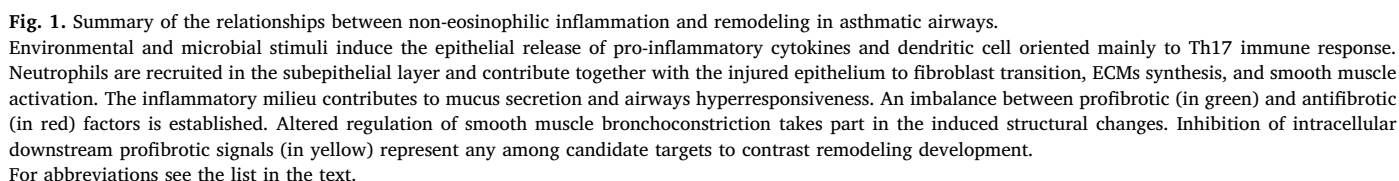
Enhanced vascularity correlates with severe asthma and is observed in biopsy from fatal asthma, but described also in mild asymptomatic asthma. The main changes in asthma vasculature are increased numbers and size of vessels per unit area, increased blood flow, vasodilatation and leakage. This changes involve microvessels below the basal lamina in the space between the muscle layer and the parenchyma [9] and contribute to increased thickness of airway walls. Increased formation of new blood vessels comes from the endothelial cells in the sub-epithelial mucosa that depends on the balance between pro and anti angiogenetic mediators.

VEGF, existing in five isoforms, can be found free soluble or sequestered by ECM. It is increased in BALF and sputum of asthmatics and stained in the submucosa of asthmatics airways and correlates with the thickness of BM. [134]. The principal sources of VEGF in lungs are fibroblasts, AECs and ASM under the stimulus of TGF- β , PGE2, IL-13, IL-4, IL-1 β , oncostatin M, and PDGF. VEGF induces the proliferation and migration of endothelial cells and their tube formation. One way for endothelial cells to activate is through integrins α v β 1 and α v β 2 that binds to matrix associated VEGF. VEGF, in addition, induce both collagen IV α -chain synthesis from podocytes and fibronectin from ASM. To be mentioned that VEGF is also chemotactic for eosinophils and linked to AHR in animal models. An antagonist of VEGFR-1 and VEGFR-2 reduce inflammatory responses in animal models yet is unknown its effect on remodeling [135]

Many ECM proteins, mainly fibronectin, collagen I and IV and thrombospondin are dysregulated in their vascular control. Tumstatin, a matrikine derived from collagen IV, as before mentioned, not only influences neutrophilic inflammation in asthma, but changes the angiogenic potential of ASM inducing the production of anti-angiogenic matrix [105].

The new microvascular formation is enhanced during prolonged allergen exposure by Th17 cells and neutralization of IL-17A reduces vascularity in the lung of OVA-challenged mice. Therefore IL-17 seems to increase the migration of ECPs to the lungs. [63]. Another chemokine critical in ECPs recruitment in lung of allergen sensitized mice are CXCL1 and CXCL2, both ligands of CXCR2. IL-17 may also act indirectly on PMVEs increasing their VEGF expression

A contributor to angiogenesis in asthma comes from tissue factor (TF), secreted by AECs after mechanical stress. Of course, the expansion of vessel component and its permeability act as an additional source for



inflammatory cells, mediators and cytokines. VEGF is also released by AECs in response to human rhinovirus, together with amphiregulin and activin A.

9. Biomarkers in airways remodeling

Novel ideas and hypotheses about airways remodeling are kinds of being taken ahold in clinical research. Discovery the good "tracers" for monitoring the severe asthma diseases in adult and pediatric patients would have a great clinical significance.

Structural airway wall changes, or airway remodeling, are a key pathological feature of asthma [2]. These changes are considered pathological because they are thought to be inappropriate to maintain normal lung function. [136].

The hallmark pathological features of asthma include airway eosinophilic inflammation and structural changes (remodeling) which are associated with an irreversible loss in lung function that tracks from childhood to adulthood. The structural airway changes are apparent in asthma even in mild disease; however, the degree of remodeling often worsens with increasing disease severity [137].

It's important to consider that not all patients have similar changes in each parameter of airway remodeling. Remodeling phenotypes should, therefore, be delineated, i.e. the contribution of each structural change (reticular basement membrane thickness, airway smooth muscle mass, angiogenesis) in an individual patient needs to be assessed. Subsequently, together with the clinical phenotype and inflammatory phenotype, the most appropriate personalized therapy can be identified for the individual. Unbiased statistical approaches that serve to identify phenotypes and endotypes need to include structural airway changes, similarly to recent analyses that have included eosinophilic and neutrophilic inflammation. Clinical trials should incorporate therefore airway biopsies and assessments of airway remodeling as outcome measures. The remodeling phenotype of noneosinophilic, noninflamed and T-helper type 2 (Th2)-low patients needs to be investigated to allow the discovery of effective molecular therapies [2].

In the era of the personalized medicine, to deliver this approach for asthma, it is important to be able to phenotype the condition in an unbiased way and to define biomarkers able to predict the course of the disease and the response to therapy. A biomarker is a measurable indicator that can evaluate either a normal or pathological biological processes or pharmacologic response to a therapeutic intervention [138]. A valid biomarker would have several key characteristics: to distinguish between disease and health with high positive and negative predictive values, to provide information about disease prognosis and clinical outcomes, to change with disease progression and "normalize" with successful treatment, to be reliable and reproducible in the clinical setting with little or no day-to-day variation, to be easy to collect in the "real-world" setting, to be quantifiable in an analytical system with well-defined performance, and to be cost-effective [139].

9.1. Periostin

Periostin is a member of the matricellular family of proteins. Matricellular proteins are defined by the ability to bind both to ECM and cell surface receptors. Periostin is known to occupy a role in airway development and alveolar epithelial repair and is notably up-regulated in infants with bronchopulmonary dysplasia [140]. Periostin influences cellular behavior via interactions with integrin receptors and can influence the production and localization of fibrogenic cytokines and growth factors [141]. In addition, it facilitates tissue remodeling and collagen crosslinking through its interaction with other ECM proteins and enzymes [142]. It is expressed in several different human tissues including the lung and, in asthma, cooperates in the deposition of ECM and proliferation of ASM cells around the airway, leading to variable narrowing of the airways with airflow obstruction.

Asthma often develops in children, but it can persist and even

develop de novo in adulthood [143]. Interestingly, periostin is one of the most highly expressed genes in asthma [144]. One study examined the production of periostin by airway epithelial cells either in asthmatic children or in cells from children that were atopic non-asthmatics versus healthy children and noted that periostin was differentially expressed in the airway epithelial cells from these three groups, with the highest expression found in the asthmatic children [145]. Moreover, its basal serum levels are physiologically relatively low (~50 ng/ml) compared to other ECMs such as fibronectin or vitronectin. Jia et al. in the Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid refractory Asthma (BOBCAT), identified serum periostin as the single best systemic biomarker of airway luminal and tissue eosinophilia in severe, uncontrolled asthmatics. Adopting 25 ng/ml serum periostin as an arbitrary cut-off, eosinophil low and eosinophil-high patients are effectively differentiated, with a positive predicted value of 93 %. Moreover, this study evidenced the superiority of serum periostin for predicting sputum and tissue eosinophilia, compared to blood eosinophils, IgE levels, YKL-40 and FeNO [146]. More recently, Kanemitsu et al., in an observational study, found that high serum periostin concentration (≥ 95 ng/ml) is the unique biomarker, among several serum markers, associated with the greater annual decline in FEV1 (at least 30 ml/year) [147]. This suggests that periostin likely plays an important role in asthmatic airway remodeling. A common endotype for this disease is one characterized by high Th2 inflammatory cytokines such as IL-4, IL-5 and IL-13 and studies support periostin as a biomarker to distinguish this Th2 endotype of asthma from Th2 low subjects [148].

Periostin also induces TGF- β signaling which can further promote ECM deposition and airway remodeling [149]. Sub-epithelial periostin promotes adherence and possibly migration of eosinophils in the lung [150]. Periostin levels have also been studied as a biomarker of immunoglobulin E (IgE)-targeted therapeutic responses. Omalizumab, a monoclonal antibody targeting IgE, has established efficacy in asthma treatment and the data support a trend towards significant clinical responses in the periostin "high" versus "low" group [151]. Corren et al. demonstrated that its higher serum levels might predict the response to Th2 target therapy with biologic agents such as an anti-IL-13 monoclonal antibody (lebrikizumab) [152]. Finally, in a recently published study, Bobolea et al. investigated the potential role of sputum periostin, more organ-targeted than serum periostin, as a biomarker of severe asthma. The authors found that sputum periostin levels are associated with persistent airflow limitation and eosinophilic inflammatory phenotype despite high-dose ICS therapy [153]. Taken together, these results show that periostin can be a useful biomarker to apply stratified medicine for severe asthma and to yield better outcomes in asthma management. In asthma, the levels of periostin are proving particularly efficacious in discriminating between different

endotypes of the disease and predicting clinical progression. [154]

Furthermore, additional carefully designed studies are needed to evaluate if periostin, alone or in combination with other more conventional biomarkers, can be utilized in better redefining current asthma phenotypes and selecting patients for emerging asthma therapeutics targeting Th2 inflammation. [155][189]

9.2. Galectins proteins (Gal3/Gal3BP)

Galectins are a family of animal lectins with different cellular and extracellular localization. These proteins bind the cell-surface and ECM glycans and, thereby, affect a variety of cellular processes and biological activities. To date, 15 galectins having a role in physiological and pathological processes like cancer, heart failure, tissue repair, and platelet aggregation have been found in mammals [156,157]. Regarding airway diseases, the effects of these proteins on the inflammatory process were analyzed for the first time in murine models. The galectin-9 role is still unclear because it was identified as a possible recruiter of eosinophil granulocytes and promoter of Th2 dominance,

but also as an IgE binding protein with anti-allergic effects able to prevent acute asthma exacerbations [158]. Another galectin with a demonstrated role in asthma is Galectin-3 (Gal-3), the only chimeric galectin found in Vertebrates, with biological activities in numerous cellular functions like cellular adhesion, growth, chemoattraction, differentiation, apoptosis and cell cycle as well as an IgE binding protein activity. Gal-3 was first discovered as an IgE-binding protein and characterized as a 32 kDa antigen (Mac-2) on the surface of murine macrophages [159]. Gal-3 is widely distributed and localizes to the extracellular space, cytoplasmic or nuclear regions.

Evaluation of Gal-3 expression in deficient and wild-type gal-3 mice with OVA-induced asthma evidenced that OVA-sensitized gal-3 (−/−) mice developed fewer eosinophils, lower goblet cell metaplasia and significantly less AHR after airway OVA challenge compared to similarly treated gal-3 (+/+) mice. Moreover, studies on Gal-3 gene therapy confirmed how it is possible to reduce eosinophils airway infiltration, AHR and tissue remodeling [160,161]. The involvement of Gal-3 in the human airways inflammatory process has been ascertained for COPD lung fibrosis and asthma. In asthma, Gal-3 expression seems to be related to the development of a specific inflammatory pattern and biological therapy outcome. Recently, Gao et al. found a significantly reduced sputum Gal-3 in patients with neutrophilic asthma [162]. Moreover, evaluating bronchial biopsies of severe asthma patients treated with omalizumab using proteomic technique, it was observed that the proteomic profile of bronchial tissue before omalizumab treatment presents a typical pattern indicative of anti-IgE treatment response. Gal-3 was expressed only in subjects with a positive bronchial morphometric analysis response to anti-IgE treatment.[190] Gal-3, having the ability to bind IgE proteins, can be considered a reliable biomarker to predict the modulation of airway remodeling and the improvement of pulmonary function in severe asthma patients before they begin omalizumab therapy [46].

Regarding ECM proteins, Gal-3 was the most stable biomarker associated with the prediction of remodeling modulation. Gal-3 gene therapy determines the inhibition of asthmatic reactions and decreases airway remodeling in animals [163] Mauri et al. showed that, before treatment, non-omalizumab responder (NOR) and omalizumab responder (OR) patients belong to distinct sub-groups differentiated by the presence of Gal-3 in bronchial tissue.

In summary, Gal-3 is a regulatory molecule acting at various stages from acute to chronic inflammation and tissue fibrogenesis. In the initial phase of allergic induction, Gal-3 stimulates inflammation through macrophage release and increased IgE production, with the final result being airway remodeling by collagen and fibronectin deposition [164]. These data suggest a possible role of Gal-3 in the omalizumab mechanism of action on remodeling, which further stresses the importance of detecting of Gal-3 as a biomarker of treatment response.

9.3. Selected bone morphogenetic proteins (BMPs)

The activation of fibroblasts, and the differentiation and secretion of increased amounts of collagen is caused mainly by TGF-β1. The sub-family of the TGF-β superfamily includes Bone Morphogenetic Proteins (BMP), Growth Differentiation Factors (GDF) and Anti-Müllerian Hormone (AMH) [165]. BMP expression was first discovered in bones but has also been found in other organs such as the bone primordia (BMP-2,4,5,7), kidneys (BMP-3,4,7), heart (BMP-2,4,6,7) and teeth (BMP-3,4,7). In addition, BMP subtypes 3, 4 and 7 have been observed in lung tissue [166]. The effects of BMP are numerous and varied: they modulate the activity of lung fibroblasts, myofibroblasts and elements of the extracellular matrix. Study results which have been obtained so far encourage to conduct in vivo and in vitro experiments with the use of animal models. Bronchial asthma is associated with dysregulation of BMP protein and BMPR receptor expression, and the constitutive level of these receptors and the changes in their expression may be used in diagnostics as predictive markers of severe asthma. [167]. The

antifibrotic activities of BMP represent a promising field for therapeutic intervention. BMP-4 and BMP-7 regulate differentially TGF-β in human fibroblasts. BMP-4 was able to downregulate TGF-β1 induced tenascin, fibronectin and collagen I and IV production, as well MMP13, whereas MMP2 was only inhibited by BMP-7 [168]. The possibility of even a partial reversal of the effects of airway remodeling can significantly translate into control of asthma symptoms, reducing the chronic effects of the prolonged disease process, extending life expectancy and improving the quality of life.

Further detailed studies of the expression and regulation of BMP proteins, their mechanisms of activation and intracellular signaling seem to be significant from the point of view of diagnostics, reflecting the development of the fields of biology and molecular biology, and the growth of gene therapy

9.4. Chitinases like protein YKL-40

Some of the most extensive functional or characterization studies of Chitinases-like Proteins (CLP) have focused around asthma and related lung diseases. CLPs are highly upregulated in the lungs of asthmatic patients and murine models of allergic airway inflammation [34,169]. The majority of studies have focused on how CLPs may be regulating adaptive Th2 responses during allergic inflammation [170]. The chitinase-like protein YKL-40 is also termed chitinase 3-like 1 (CHI3L1). It is produced at sites of inflammation in many cells and is secreted from macrophages, epithelial cells and smooth muscle cells. YKL-40 binds to ubiquitously expressed chitin but lacks chitinase activity. Previous studies have demonstrated that YKL-40 was associated with pathologic conditions characterized by aberrant cell growth, tissue inflammation, subepithelial basal membrane thickening and remodeling [171]. A recent study suggested that serum YKL-40 levels were significantly elevated in patients with asthma compared to controls. This result indicates that high levels of serum YKL-40 may be a biological characteristic of asthma exacerbation [172,173]. YKL-40 mediates airway inflammation, and serum levels are associated with asthma severity. By cluster analysis, high YKL-40 levels were associated with a longer disease, severe airflow obstruction, and near-fatal asthma. The airway transcriptome analysis showed activation of non-type-2 inflammatory pathways [174].

However, YKL-40 expression positively correlates to neutrophilic and IL-17-driven inflammation in asthmatic patients, suggesting CLPs may also be important for non-Th2 asthma phenotypes that are often strongly associated with innate immune responses. [175]

Lai et al. has demonstrated that patients with elevated levels of YKL-40 had significantly greater corticosteroid use than patients with lower levels, which suggests that high levels of serum YKL-40 may be refractory to current asthma treatments. Future prospective studies are required to determine the role of YKL-40 in patients with severe therapy-resistant asthma and will assess whether YKL-40 gene expression is directly controlled by corticosteroids [176].

9.5. Vitamin D

The exact mechanisms underlying the association between vitamin D and lung function are not completely understood. However, some hypothesized mechanisms might explain this relationship. One possible explanation is the role vitamin D plays in airway remodeling [177]

As the results of the current study have shown similar associations for lung function measurements pre- and post-bronchodilation, structural, and not functional, changes in the lung are suggested, which would support the hypothesis of vitamin D influencing the airway remodeling. Another hypothesis might be related to the influence of vitamin D on the development and modulation of the immune system. For instance, the active vitamin D metabolite can lead to an inhibition of the activation and proliferation of T-lymphocytes. It has been shown that Th1-associated cytokine production is inhibited, whereas Th2

responses could be inhibited as well as enhanced. Both Th1 and Th2 cells are involved in asthma by producing pro- and anti-inflammatory cytokines. Furthermore, vitamin D might support regulatory T-cells, which inhibit airway hyperresponsiveness and airway inflammation [178]. Lung development, growth, and maturation as well as surfactant secretion might also be affected by vitamin D via its receptors present in fetal type II alveolar cells. The study by Zosky et al. [179] has shown that vitamin D-deficient mice have a significantly reduced lung volume, in terms of thoracic gas volume, compared to vitamin D-replete mice. Furthermore, an influence of vitamin D on the alveolar epithelial-mesenchymal interactions was observed in the study by Sakurai et al. [180]. The active vitamin D metabolite inhibits apoptosis and thus increases alveolar type II cell proliferation. Besides surfactant synthesis, the production of interstitial fibers, i.e. collagen type I alpha 1, is affected by vitamin D deficiency. These experimental studies support the hypothesis that vitamin D affects the alveolar region of the lung, causing changes in lung volume rather than airflow limitations, which is in line with our and other findings that FVC is mainly affected by serum 25(OH)D concentrations.

An interesting relationship that may provide therapeutic potential is between airway smooth muscle, steroid resistance, and serum vitamin D levels. Saglani et al. have demonstrated a negative relationship between airway smooth muscle mass and serum vitamin D levels in children with severe therapy Resistant Asthma (STRA) [168]. Moreover, lower vitamin D levels were associated with lower lung function and increased asthma symptoms. Children with low serum vitamin D also had lower bronchoalveolar (BAL) levels of the anti-inflammatory mediator IL-10. When peripheral blood mononuclear cells (PBMCs) from children with STRA were stimulated with dexamethasone, the release of IL-10 was significantly lower than that from control PBMCs. However, the addition of both dexamethasone and vitamin D3 resulted in a significant increase in IL-10 secretion. These data suggest vitamin D enhances steroid sensitivity in STRA, and supplementation may not only help to improve steroid responsiveness, but also may impact airway smooth muscle remodeling. A further interesting mechanism between vitamin D and steroid resistance in STRA involves IL-33.

Vitamin D concentrations are positively associated with volume-related lung function parameters pre- and post-bronchodilation, suggesting structural changes in peripheral airways. [181]

10. Conclusions

The comprehension of the immunological mechanisms at the basis of airway remodeling is still one of the most challenging goals that scientists and clinicians must reach for an exhaustive solution of the unmet needs in asthma cure.

Numerous factors intrinsic to the phenomenon of airway remodeling are evident limits both for the basic research and for the clinical application [182]. Among these factors, the lack of standardized methodology in the assessment of the remodeling features and the identification of specific clinical outcomes often taper the relevance of the findings. Moreover, assuming the accuracy of them, many of the physiopathological evidence as far as reported needs to be clarified [1]. First remodeling is associated with both severe asthma and newly diagnosed disease in children and adults. Thus the association between remodeling and the evolution to fixed airflow obstruction is not generalized. As a consequence, remodeling possesses a longitudinal and progressive nature implying a research approach different from the cross-sectional one [183]. Another crucial point is that the anatomical distribution of airway structural changes could extend also to small airways, yet not easy to demonstrate.

The experimental studies reported in this review point out a complex and not univocal relationship between airway remodeling and inflammation. As a consequence the currently available anti-asthma treatments targeted to inflammatory mediators seems not resolute over many of the remodeling end-points. Corticosteroids do not seem to

reverse significant structural asthmatic changes, except for collagen BM deposition [184]. The observed effect due to anti-IL5 treatment on RBM thickness was not followed by convincing results on lung function [185]. The ability of omalizumab, the anti-IgE antibody, to decrease collagen deposition and RBM thickness can be associated with its contribution to the persistent reversal of airway obstruction in only a portion of patients with severe allergic asthma [46].

In addition, is not yet known which inflammatory pathway contributes mainly to the initiation, maintenance, and progression of remodeling or can be assumed to be the mainstream for either AHR or fixed obstruction.[186] Asthma is recognized to be a heterogeneous disease incorporating different clinical and inflammatory phenotypes and endotypes [187] as revealed by both biased and unbiased approaches. Notwithstanding the above remarks, it is conceivable that a more comprehensive knowledge of the remodeling phenomenon will come from the identification of phenotypes and endotypes including structural airway changes and assessments of airway remodeling outcome measures.

We can expect that not all asthmatic patients show the same degree of remodeling features and that different cell types may have different roles in the remodeling determinism according to exposures, (atopy, infection, environment, smoke), duration of the disease, age-onset, steroid-resistance.

In this review we propose point by point how cells of the natural and adaptive immune system orchestrate in synergy the development of the structural hallmarks of airway remodeling. From this systematic approach emerge both associations between assumable phenotypes of remodeling and immune molecular pathways as well as surrogate biomarkers.

The overall picture of either the noneosinophilic and the Th2 driven inflammation pathways associated with remodeling is reported in Figs. 1 and 2 respectively.

The complex process of airways remodeling still requires further investigation because many dynamics are unclear, the tools for a detailed analysis of the phenomenon are still insufficient, above all in the application of personalized medicine systems

The need for finding a panel of cellular and molecular biomarkers is useful to monitor the history and development of asthma. Basic and clinical research in remodeling should actively collaborate and integrate to recognize combined biomarker panels and to identify phenotypes and endotypes of patients eligible for advanced treatments.

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Declaration of Competing Interest

None.

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