Airway Structural Changes in Asthma

Subjects: Pathology

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Increased airway wall thickness and remodeling of bronchial mucosa are characteristic of asthma and may arise from altered integrin signaling on airway cells. Here, we analyzed the expression of β1-subfamily integrins on blood and airway cells (flow cytometry), inflammatory biomarkers in serum and bronchoalveolar lavage, reticular basement membrane (RBM) thickness and collagen deposits in the mucosa (histology), and airway geometry (CT-imaging) in 92 asthma patients (persistent airflow limitation subtype: n=47) and 36 controls. Persistent airflow limitation was associated with type-2 inflammation, elevated soluble α2 integrin chain, and changes in the bronchial wall geometry. Both subtypes of asthma showed thicker RBM than control, but collagen deposition and epithelial α1 and α2 integrins staining were similar. Type-I collagen accumulation and RBM thickness were inversely related to the epithelial expression of the α2 integrin chain. Expression of α2β1 integrin on T-cells and eosinophils was not altered in asthma. Collagen I deposits were, however, more abundant in patients with lower α2β1 integrin on blood and airway CD8+ T-cells. Thicker airway walls in CT were associated with lower α2 integrin chain on blood CD4+ T-cells and airway eosinophils. Our data suggest that α2β1 integrin on inflammatory and epithelial cells may protect against airway remodeling advancement in asthma.

Keywords: asthma ; airway remodeling ; computed tomography ; biomarkers ; histology

1. Introduction

Airway remodeling refers to structural and functional changes in bronchial walls caused by inflammation and repeated cycles of injury and repair [1]. In asthma, it is characterized by structural and functional alterations of airway epithelium and subepithelial fibrosis, with thickening of the basement membrane and increased deposition of extracellular matrix (ECM) proteins in submucosa being the most prominent features [2]. Thickening of the basement membrane occurs mainly in the lamina reticularis layer named reticular basement membrane (RBM), which is composed of collagen fibers (mostly type III) produced primarily by underlying connective tissue cells [2][3]. There are more than twenty different subtypes of collagen, of which collagens I and III constitute the structural framework of lungs. In the asthmatic airways, collagen deposits accumulate in the basement membrane in a disorganized and fragmented form [4]. In contrast, type IV collagen is present mainly in the basal lamina, where it forms the central platform for anchoring epithelial cells [5].

Increased smooth muscle mass and increased airway wall stiffness is another consistent trait of airway remodeling that correlates with impaired lung function in asthma [6][7]. However, it may also protect against exaggerated responses to allergens and other inflammatory stimuli, preventing immediate bronchoconstriction [8]. Progression of remodeling has been linked with chronic airway inflammation, although the causal relationship is uncertain [9]. Nevertheless, repeated mechanical stress may cause bronchial wall structural changes even in the absence of inflammation [10].

Various features of airway remodeling, such as loss of epithelial cells and mucus cell hyperplasia, RBM thickening, and smooth muscle hypertrophy, can be described using a histological examination of airway mucosa [11]. However, emerging non-invasive methods, including lung computed tomography (CT) imaging, provide important remodeling measures that might be useful in quantifying bronchial wall thickening in a standardized and more comprehensive way [12][13].

Integrins are a large family of transmembrane glycoproteins that mediate cell–cell and cell–ECM interactions, and thus are involved in a broad range of cellular processes, including cell adhesion, migration, and proliferation [14]. Each integrin subfamily is characterized by a common β subunit and non-covalently associated variable α chain. The β_1-subfamily includes, among others, integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$, and $\alpha_{11}\beta_1$ that are characterized by high-affinity binding to GFOGER-like motifs in collagen. Integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$, which show a preference for binding to type IV and I collages, respectively, are essential members within that subset [14]. They are expressed on various cells, including vascular and epithelial cells, fibroblasts, and activated T-cells, responsible for the cell interactions with collagen fibers in basement membranes and ECM [15][16]. Therefore, both may be involved in asthma airway remodeling pathology. In an early report, Schuliga et al. [17] showed that interaction of airway smooth muscle cells with type I collagen via $\alpha_2\beta_1$ integrin potentiated conversion of plasminogen into plasmin with subsequent degradation of ECM proteins. Furthermore, it has been demonstrated that in

vitro inhibition of $\alpha_2\beta_1$ integrin on fibroblasts enhanced their proliferation together with excessive lung ECM deposition and tissue fibrosis [18], supporting the concept of the potential antifibrotic role of $\alpha_2\beta_1$ integrin in airways.

A growing body of evidence suggests that T-cell interactions with ECM proteins in perivascular tissues are essential for regulating the inflammatory response [19]. Furthermore, collagen-binding integrins not involved in cell migration occurred to be crucial costimulatory molecules of effector T cells [19][20]. Moreover, it has been demonstrated that $\alpha_2\beta_1$ integrin may promote the survival of effector T cells by inhibiting Fas-induced apoptosis [21]. Although naïve T cells express very low levels of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins, they become abundant upon in vitro activation [19]. Asthma is an airway disease with a locally limited inflammatory response. However, increased blood levels of inflammatory cytokines and signs of coagulation pathway activation suggest accompanying low-grade systemic inflammation [22][23]. Thus, activated immune and effector cells, such as T-cells and eosinophils, although essential for airway site, may also be found in the systemic circulation likely primed in airways or by circulating inflammatory cytokines.

2. Clinical Characteristics and Airway Inflammatory Signature in Asthma Patients with Persistent Airflow Limitation

We analyzed 92 adult, non-smoking asthma patients and 36 controls. Among asthmatics, 47 subjects were characterized by persistent airflow limitation, while 45 had normal spirometry before or after a bronchodilator (non-persistent airflow limitation subtype). All three analyzed groups were similar in demographic variables, including body mass index (BMI), and past smoking, although asthma patients with persistent airflow limitation were older than the remaining asthmatics (Table 1). The studied asthma subtypes did not differ in disease duration and severity, staged according to the Global Initiative for Asthma (GINA) guidelines [24]. Atopy was more frequent in asthmatics. Other comorbidities were equally prevalent in all three analyzed groups, except for gastroesophageal reflux disease (GERD), which was more prevalent in the control individuals (Table 1).

Table 1. Demographic and clinical characteristics of the subjects studied.

Non-Persistent Airflow Limitation *n* = 45	Persistent Airflow Limitation *n* = 47	Control *n* = 36	*p*-Value Non-Persistent vs. Persistent Limitation	*p*-Value Non-Persistent Limitation vs. Control	*p*-Value Persistent Limitation vs. Control
Demographic variables					
Age, years	52 (41–59)	58 (52–65)	55 (45–65)	0.004	0.07	0.27
Male gender, *n* (%)	10 (22)	16 (34)	5 (14)	0.15	0.5	0.07
Body mass index, kg/m^2	27.8 (24.8–30.8)	26.4 (23.5–31.6)	27.3 (23.0–27.9)	0.53	0.13	0.95
Smoking history						
Past smoking, *n* (%)	13 (29)	15 (32)	12 (33)	0.93	0.85	0.92
Pack-years of smoking	0 (0–7)	0 (0–8)	0 (0–4)	0.85	0.84	0.9
Comorbidities						
Atopy, *n* (%)	27 (60)	23 (49)	6 (17)	0.39	0.0002	0.005
GERD, *n* (%)	16 (36)	22 (47)	23 (64)	0.38	0.02	0.19
Arterial hypertension, *n* (%)	18 (40)	28 (60)	15 (42)	0.09	0.94	0.16
Diabetes mellitus, *n* (%)	6 (13)	12 (26)	3 (8)	0.23	0.72	0.08
Hypercholesterolemia, *n* (%)	9 (20)	16 (34)	6 (17)	0.2	0.92	0.13
Coronary heart disease, *n* (%)	2 (4)	5 (11)	2 (6)	0.47	0.77	0.67
Asthma-related variables						
Asthma duration, years	11.5 (5–19.5)	10 (7–20)		0.86		

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Asthma severity (GINA): persistent mild, *n* (%) persistent moderate, *n* (%) persistent severe, *n* (%)	 8 (18) 22 (49) 15 (33)	 7 (15) 15 (32) 25 (53)		0.14		
Asthma treatment: Inhaled corticosteroids, *n* (%) Long-acting β2-agonists, *n* (%) Montelukast, *n* (%) Theophylline, *n* (%) Oral corticosteroids, *n* (%)	 45 (100) 31 (69) 9 (20) 4 (9) 8 (18)	 47 (100) 42 (89) 4 (9) 10 (21) 15 (32)		0.17		
Spirometry results						
FEV_1 before bronchodilator, L	2.79 ± 0.76	1.79 ± 0.8	2.71 ± 0.75	<0.001	0.65	<0.001
FEV_1 before bronchodilator, % of the predicted value	100.3 (89.5–111.1)	66.7 (54.1–80.6)	110.9 (106.8–114.7)	<0.001	<0.001	<0.001
FEV_1 after bronchodilator, L	2.92 ± 0.73	2.07 ± 0.95	2.84 ± 0.79	<0.001	0.66	<0.001
FEV_1 after bronchodilator, % of the predicted value	103.8 (96.4–116.5)	79.2 (62.8–87.2)	116 (112.1–122.3)	<0.001	<0.001	<0.001
FEV_1/VC (before bronchodilator)	73.3 (67.8–78.18)	59.1 (51.7–63.8)	74.84 (73.23–78.38)	<0.001	0.16	<0.001
FEV_1/VC (after bronchodilator)	76.99 (73.05–81.88)	65.4 (54.5–68.6)	79.33 (77.25–80.38)	<0.001	0.25	<0.001

Table 1 footnote. Categorical variables are presented as numbers (percentages), continuous variables as median and interquartile range, or mean and standard deviation, as appropriate. Abbreviations: GERD—Gastroesophageal reflux disease, GINA—Global Initiative for Asthma, FEV_1—Forced expiratory volume in 1 s, VC—Vital capacity, L-liter, *n*—number. Statistics: Mann–Whitney U-test or unpaired t-test, as appropriate.

Asthma patients showed higher serum IgE and increased red blood cell, lymphocyte, and monocyte counts compared to controls (Table 2). In turn, white blood cells and neutrophils were elevated in asthma patients with persistent airflow limitation than in other groups. Furthermore, asthma patients with persistent airflow limitation were characterized by elevated type-2 (T2) inflammatory biomarkers, such as blood and BAL eosinophilia, and serum periostin when comparing the remaining asthmatics (Table 2). Serum levels of a disintegrin and metalloproteinase domain-containing protein (ADAM)-33 were also increased in the asthma subtype with persistent airflow limitation (Table 2).

Table 2. Laboratory variables.

Reference Range	Non-Persistent Airflow Limitation *n* = 45	Persistent Airflow Limitation *n* = 47	Control *n* = 36	*p*-Value Non-Persistent vs. Persistent Limitation	*p*-Value Non-Persistent Limitation vs. Control	*p*-Value Persistent Limitation vs. Control
Basic laboratory tests						
Red blood cells, 10^6/μL	4–5	4.65 ± 0.4	4.7 ± 0.5	4.48 ± 0.4	0.68	0.048	0.03
White blood cells, 10^3/μL	4–10	6.26 (5.43–7.33)	7.44 (6.39–9.25)	5.44 (5.16–7.08)	<0.001	0.07	<0.001
Neutrophils, 10^3/μL	1.8–7.7	3.1 (2.7–4.1)	3.7 (2.9–4.8)	3.3 (2.9–3.6)	0.049	0.76	0.04

Reference Range	Non-Persistent Airflow Limitation *n* = 45	Persistent Airflow Limitation *n* = 47	Control *n* = 36	*p*-Value Non-Persistent vs. Persistent Limitation	*p*-Value Non-Persistent Limitation vs. Control	*p*-Value Persistent Limitation vs. Control	
Lymphocytes, 10^3/µL	1–4.5	1.94 (1.58–2.43)	2.2 (1.58–2.61)	1.65 (1.44–2.08)	0.5	0.03	0.03
Monocytes, 10^3/µL	0.1–0.8	0.57 (0.49–0.74)	0.71 (0.53–0.9)	0.49 (0.41–0.62)	0.02	0.006	<0.001
Blood platelets, 10^3/µL	140–400	223 (193–247)	225 (191–265)	228 (189–246)	0.78	0.98	0.85
Asthma and inflammatory biomarkers (blood)							
Eosinophilia/µL	40–450	230 (130–310)	400 (180–680)	110 (70–170)	0.009	<0.001	<0.001
Immunoglobulin E, IU/mL	0–100	90 (26–400)	88 (43–511)	23 (18–48)	0.6	<0.001	<0.001
C-reactive protein, mg/L	0–5	1.64 (0.53–8)	4.53 (0.58–9.38)	1.78 (0.89–2.29)	0.39	0.28	0.008
Fibrinogen, g/L	1.8–3.5	3.1 (2.8–3.5)	3.5 (3.2–4.2)	2.9 (2.3–3.7)	0.03	0.11	0.002
Periostin, ng/mL	0.29–0.61 §	0.28 (0.24–0.33)	0.38 (0.31–0.51)	0.37 (0.36–0.45)	0.01	0.001	0.85
Interleukin 6, pg/mL	0.005–1.432 §	0.72 (0.43–1.19)	1.09 (0.47–2.38)	0.57 (0.43–0.97)	0.14	0.29	0.03
Interleukin 10, pg/mL	0.163–1.022 §	0.6 (0.22–1.06)	0.55 (0.35–0.89)	0.43 (0.2–0.76)	0.95	0.17	0.1
Interleukin 12 (p70), pg/mL	0.005–2.618 §	0.005 (0.005–1.2)	0.005 (0.005–1.25)	0.005 (0.005–0.33)	0.7	0.13	0.26
ADAM-33, ng/mL	0.083–2.257 §	0.73 (0.2–1.29)	1.32 (0.33–2.37)	0.41 (0.13–1.5)	0.01	0.65	0.007
Circulating integrin subunits							
α_1 integrin, ng/mL	6.45–103.67 §	17.32 (6.88–52.4)	32.7 (14.7–55.7)	24.1 (8.90–76.5)	0.14	0.21	0.83
α_2 integrin, ng/mL	7.79–36.19 §	15.5 (9.7–25.5)	22.9 (15–39)	20.5 (11.7–26.5)	0.03	0.25	0.21
Asthma and inflammatory biomarkers (bronchoalveolar lavage fluid)							
Periostin, ng/mL	0.1–1.15 §	0.86 (0.8–0.99)	0.81 (0.72–0.95)	0.8 (0.51–0.88)	0.34	0.17	0.49
Eosinophils, %	0–1 #	0.5 (0–1)	1 (0.1–3)	0.1 (0–1)	0.02	0.62	0.006

3. Asthma Is Characterized by Decreased Expression of α_4 and β_1 on Circulating Inflammatory Cells and Increased Expression of α_1 Integrin Chain

First, we analyzed the expression of β_1-subfamily integrins on blood and BAL inflammatory cells in asthma and control individuals. Surprisingly, blood $CD8^+$ T-cells and eosinophils in asthma were characterized by lower expression of α_4 and β_1 integrin chains (Figure 1). Furthermore, circulating and airway $CD4^+$ T-cells showed lower expression of β_1 integrin chains, whereas BAL $CD4^+$ T-cells additionally had increased expression of α_1 (Figure 1a). The α_1 integrin chain was also present on a higher percentage of blood eosinophils and blood $CD8^+$ T-cells.

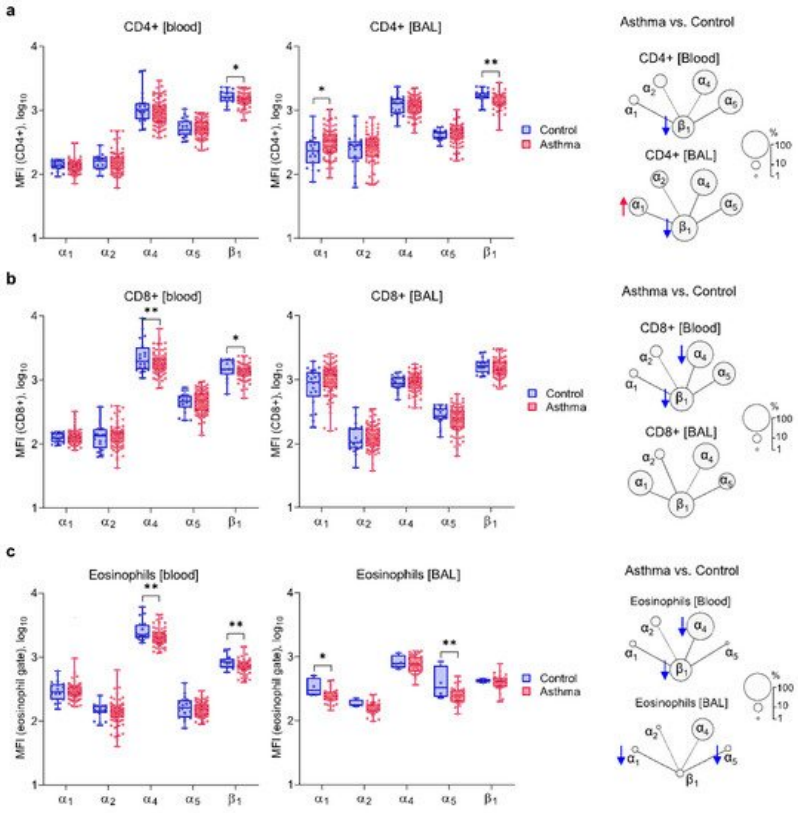

Figure 1. Surface expression of integrin chains on T-cells and eosinophils in asthma and control subjects. Median fluorescent intensities (MFI) of the studied integrin chains in different compartments (on the left) and diagram summarizing the differences in the expression between the two analyzed groups (on the right, circle areas reflect the percentage of cells positive for a given integrin). (**a**,**b**) Asthma patients showed higher expression of α_1 integrin chain on bronchoalveolar lavage (BAL) $CD4^+$ T-cells (red arrow) and decreased expression of β_1 (blue arrow) on $CD4^+$ blood and BAL and $CD8^+$ blood T-cells and lower expression of α_4 on blood $CD4^+$ T cells. (**c**) Lower expression of surface α_4 and β_1 integrin chains on blood eosinophils and α_1 and α_5 on BAL eosinophils in asthma. Data presented as medians and range (T-cells: blood n = 108, BAL n = 101; eosinophils: blood n = 103, BAL n = 35; β_1 was measured in 78% of samples). Statistics: ANCOVA with adjustment for age, sex, and BMI: * $p < 0.05$, ** $p < 0.01$.

4. Similar Expressions of α_1 and α_2 Integrin Chains on Blood and BAL Inflammatory Cells of Both Asthma Subsets

Next, we compared the expression of studied integrin subunits in persistent vs. non-persistent airflow limitation patients and controls. In comparison to the control group, patients with persistent airflow limitation showed lower expression of α_4 and β_1 on both blood T-cell subsets and eosinophils and decreased β_1 on BAL T-cells. Additionally, they were characterized by increased expression of α_1 integrin chain on BAL $CD4^+$ T cells, albeit once again only compared to the controls (Figure 2). They also had lower α_4 on BAL T-cells than those with non-persistent airflow limitation (Figure 2a,b). Compared to controls, the latter group had elevated α_1 on BAL $CD4^+$ T cells (Figure 2a).

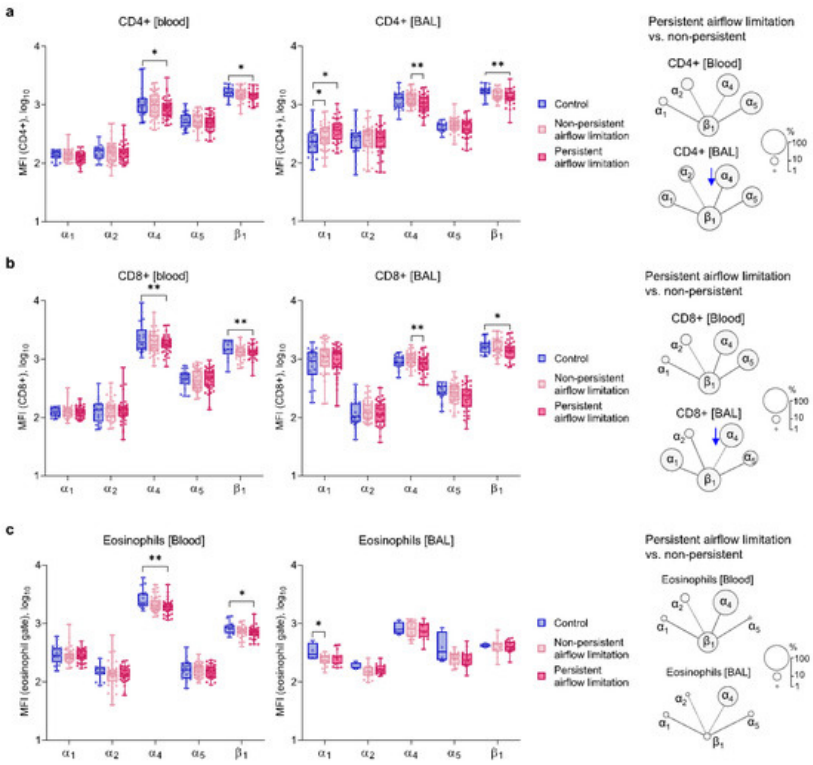

Figure 2. Surface expression (median fluorescence intensity [MFI]) of integrin chains on T-cells and eosinophils in asthma patients with persistent vs. non-persistent airflow limitation and controls. (**a**,**b**) Patients with persistent airflow limitation showed lower expression of α_4 and β_1 on both blood T-cell subsets, decreased β_1 on both BAL T-cell subsets, and increased α_1 on BAL $CD4^+$ T-cells but only compared to controls. They also had lower α_4 on both BAL T-cell subsets comparing the non-persistent airflow limitation group (blue arrows in the diagram on the right). (**c**) Blood eosinophils of persistent airflow limitation patients had lower α_4 and β_1 than controls and no difference compared to the non-persistent airflow limitation subgroup. Data are presented as medians and range (T-cells: blood n = 108, BAL n = 101; eosinophils: blood n = 103, BAL n = 35; β_1 was measured in 78% of samples). Statistics: ANCOVA with adjustment for age, sex, and BMI: * $p < 0.05$, ** $p < 0.01$.

Blood eosinophils in patients with persistent airflow limitation also had a lower expression of α_4 and β_1 than in controls; they also did not differ from the non-persistent airflow limitation subgroup (Figure 2c).

References

1. Busse, W.W.; Lemanske, R.F. Asthma. N. Engl. J. Med. 2001, 344, 350–362.
2. Trejo Bittar, H.E.; Yousem, S.A.; Wenzel, S.E. Pathobiology of Severe Asthma. Annu. Rev. Pathol. Mech. Dis. 2015, 10, 511–545.
3. Ito, J.T.; Lourenço, J.D.; Righetti, R.F.; Tibério, I.F.L.C.; Prado, C.M.; Lopes, F.D.T.Q.S. Extracellular Matrix Component Remodeling in Respiratory Diseases: What Has Been Found in Clinical and Experimental Studies? Cells 2019, 8, 342.
4. Mostaço-Guidolin, L.B.; Osei, E.T.; Ullah, J.; Hajimohammadi, S.; Fouadi, M.; Li, X.; Li, V.; Shaheen, F.; Yang, C.X.; Ch u, F.; et al. Defective Fibrillar Collagen Organization by Fibroblasts Contributes to Airway Remodeling in Asthma. Am. J. Respir. Crit. Care Med. 2019, 200, 431–443.
5. Halfter, W.; Oertle, P.; Monnier, C.A.; Camenzind, L.; Reyes-Lua, M.; Hu, H.; Candiello, J.; Labilloy, A.; Balasubramani, M.; Henrich, P.B.; et al. New Concepts in Basement Membrane Biology. FEBS J. 2015, 282, 4466–4479.
6. Hirota, N.; Martin, J.G. Mechanisms of Airway Remodeling. Chest 2013, 144, 1026–1032.
7. Fehrenbach, H.; Wagner, C.; Wegmann, M. Airway Remodeling in Asthma: What Really Matters. In Cell and Tissue Re search; Springer: Cham, Switzerland, 2017; pp. 551–569.
8. James, A.L.; Wenzel, S. Clinical Relevance of Airway Remodelling in Airway Diseases. Eur. Respir. J. 2007, 30, 134–1 55.
9. James, A.L.; Bai, T.R.; Mauad, T.; Abramson, M.J.; Dolhnikoff, M.; McKay, K.O.; Maxwell, P.S.; Elliot, J.G.; Green, F.H. Airway Smooth Muscle Thickness in Asthma Is Related to Severity but Not Duration of Asthma. Eur. Respir. J. 2009, 3 4, 1040–1045.

10. Grainge, C.L.; Lau, L.C.K.; Ward, J.A.; Dulay, V.; Lahiff, G.; Wilson, S.; Holgate, S.; Davies, D.E.; Howarth, P.H. Effect of Bronchoconstriction on Airway Remodeling in Asthma. N. Engl. J. Med. 2011, 364, 2006–2015.
11. Bazan-Socha, S.; Buregwa-Czuma, S.; Jakiela, B.; Zareba, L.; Zawlik, I.; Myszka, A.; Soja, J.; Okon, K.; Zarychta, J.; Kozlik, P.; et al. Reticular Basement Membrane Thickness Is Associated with Growth-and Fibrosis-Promoting Airway Transcriptome Profile-Study in Asthma Patients. Int. J. Mol. Sci. 2021, 22, 998.
12. Kozlik, P.; Zuk, J.; Bartyzel, S.; Zarychta, J.; Okon, K.; Zareba, L.; Bazan, J.G.; Kosalka, J.; Soja, J.; Musial, J.; et al. The Relationship of Airway Structural Changes to Blood and Bronchoalveolar Lavage Biomarkers, and Lung Function Abnormalities in Asthma. Clin. Exp. Allergy 2020, 50.
13. Choi, S.; Hoffman, E.A.; Wenzel, S.E.; Castro, M.; Fain, S.; Jarjour, N.; Schiebler, M.L.; Chen, K.; Lin, C.-L.; National Heart, Lung and Blood Institute's Severe Asthma Research Program. Quantitative Computed Tomographic Imaging-Based Clustering Differentiates Asthmatic Subgroups with Distinctive Clinical Phenotypes. J. Allergy Clin. Immunol. 2017, 140, 690–700.e8.
14. Bazan-Socha, S.; Bukiej, A.; Marcinkiewicz, C.; Musial, J. Integrins in Pulmonary Inflammatory Diseases. Curr. Pharm. Des. 2005, 11, 893–901.
15. Zeltz, C.; Gullberg, D. The Integrin-Collagen Connection—A Glue for Tissue Repair? J. Cell Sci. 2016, 129, 653–664.
16. Bertoni, A.; Alabiso, O.; Galetto, A.; Baldanzi, G. Integrins in T Cell Physiology. Int. J. Mol. Sci. 2018, 19, 485.
17. Schuliga, M.; Harris, T.; Stewart, A.G. Plasminogen Activation by Airway Smooth Muscle Is Regulated by Type I Collagen. Am. J. Respir. Cell Mol. Biol. 2011, 44, 831–839.
18. Xia, H.; Seeman, J.; Hong, J.; Hergert, P.; Bodem, V.; Jessurun, J.; Smith, K.; Nho, R.; Kahm, J.; Gaillard, P.; et al. Low α2β1 Integrin Function Enhances the Proliferation of Fibroblasts from Patients with Idiopathic Pulmonary Fibrosis by Activation of the β-Catenin Pathway. Am. J. Pathol. 2012, 181, 222–233.
19. Boisvert, M.; Chetoui, N.; Gendron, S.; Aoudjit, F. Alpha2beta1 Integrin Is the Major Collagen-Binding Integrin Expressed on Human Th17 Cells. Eur. J. Immunol. 2010, 40, 2710–2719.
20. Rao, W.H.; Hales, J.M.; Camp, R.D.R. Potent Costimulation of Effector T Lymphocytes by Human Collagen Type I. J. Immunol. 2000, 165, 4935–4940.
21. Gendron, S.; Couture, J.; Aoudjit, F. Integrin A2β1 Inhibits Fas-Mediated Apoptosis in T Lymphocytes by Protein Phosphatase 2A-Dependent Activation of the MAPK/ERK Pathway. J. Biol. Chem. 2003, 278, 48633–48643.
22. Bazan-Socha, S.; Mastalerz, L.; Cybulska, A.; Zareba, L.; Kremers, R.; Zabczyk, M.; Pulka, G.; Iwaniec, T.; Hemker, C.; Undas, A. Prothrombotic State in Asthma Is Related to Increased Levels of Inflammatory Cytokines, IL-6 and TNFα, in Peripheral Blood. Inflammation 2017, 40, 1125–1235.
23. Kuczia, P.; Zuk, J.; Iwaniec, T.; Soja, J.; Dropinski, J.; Malesa-Wlodzik, M.; Zareba, L.; Bazan, J.G.; Undas, A.; Bazan-Socha, S. Citrullinated Histone H3, a Marker of Extracellular Trap Formation, Is Increased in Blood of Stable Asthma Patients. Clin. Transl. Allergy 2020, 10, 31.
24. Global Initiative for Asthma—Global Initiative for Asthma—GINA. Available online: (accessed on 26 September 2018).