Overlap Between Asthma and COPD: Where the Two Diseases Converge

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INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are traditionally recognized as distinct diseases, with some clearly separate characteristics. Asthma originates in childhood, is associated with allergies and eosinophils, and is best treated by targeting inflammation, whereas COPD occurs in adults who smoke, involves neutrophils, and is best treated with bronchodilators and the removal of risk factors. However, the distinction between the two is not always clear. Patients with severe asthma may present with fixed airway obstruction, and patients with COPD may have hyperresponsiveness and eosinophilia. Recognizing and understanding these overlapping features may offer new insight into the mechanisms and treatment of chronic airway inflammatory diseases.

Key Words: Asthma; COPD; overlap; exacerbation; remodeling

WHAT IS THE OVERLAP BETWEEN ASTHMA AND COPD?

A patient who has features of more than one condition exhibits an overlap syndrome.6 The pathogenesis of overlapping asthma and COPD may be mediated by inflammatory/immune mechanisms and/or structural alterations. The clinical recognition of overlapping asthma and COPD requires an assessment of increased variability of airflow and incompletely reversible airflow obstruction.6 Numerous studies have documented the presence of partial reversibility after short-term and long-term bronchodilator administration in patients with COPD.7,8 Current guidelines emphasize a fixed or irreversible component to airflow obstruction in some patients with asthma.9,10 Thus, the use of phenotypic characteristics (e.g., symptoms, allergy, bronchi-
The distinction between the inflammatory profiles of asthma and COPD may be blurred under certain circumstances. Classically, asthmatic airways show a CD4+ lymphocyte-, eosinophil-, and macrophage-rich inflammatory response, whereas prominent increases in CD8+ T cells, neutrophils, and macrophages are seen in the bronchioles and alveoli in COPD. However, compared with mild and moderate asthmatics, severe asthmatics or asthmatics who smoke show higher numbers of neutrophils in bronchoalveolar lavage fluid and biopsies. Conversely, in COPD patients, especially those with acute disease exacerbations, tissue eosinophilia is common and is associated with a favorable response to steroid therapy.

In asthmatics, there is a predominance of Th2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, and upregulation of chemokines, including regulated on activation, normal T-cell-expressed and secreted (RANTES), eotaxins, and monocyte chemoattractant protein-1. In contrast, Th1-dominated responses such as enhanced production of interferon-γ by CD8+ cells have been documented in COPD patients. Additionally, the main inflammatory mediators involved in the pathogenesis of tissue inflammation in COPD are the neutrophil chemokine IL-8, leukotriene B4, IL-1, and tumor necrosis factor-α. However, in some COPD subjects, the levels of tumor necrosis factor-α indicated the presence of asthma. Conversely, allergic inflammatory mediators such as IL-4, IL-5, and IL-13 were produced, particularly during exacerbations.

Alveolar inflammation and the development of lung emphysema are major characteristics of COPD. The distal lung, including the alveolar parenchyma, is an important site of inflammation in asthma, although asthma is classically considered as a chronic inflammatory disease of the airways. Remodeling of various structural components such as airway epithelium, airway smooth muscle, vessel, mucous gland, and extracellular matrix is prominent in asthmatic airways. The pathological changes within the airways that are associated with asthma and COPD are similar. Based on the complexities of the relationships among the clinico-pathobiological features of the two diseases, the ‘Dutch hypothesis’ was proposed and updated. This hypothesis suggests that asthma and COPD are different expressions of a single disease entity. In contrast, the ‘British hypothesis’ states that asthma and COPD are separate clinical entities with similar symptoms. The general consensus under either hypothesis is that similar mechanisms are involved in the pathogenesis of both diseases.

**EPISODIC (TEMPORAL) CONVERGENCE OF ASTHMA AND COPD**

**Exacerbation/infection: Changes in inflammatory features and cytokine profiles**

Exacerbations of asthma and COPD are clinically significant events. They are frequently triggered by viral infections of the airways and are associated with a decline in lung function and symptomatic aggravation. During exacerbation, airway inflammation becomes more exaggerated than in the mild and stable disease states, and the inflammation pattern changes. Neutrophil recruitment is a prominent feature of acute exacerbation of chronic asthma, probably owing to respiratory tract infection by viruses. Furthermore, neutrophilic inflammation in the absence of eosinophils is largely present in sudden-onset fatal asthma, and neutrophil numbers are highly elevated in status asthmaticus. Thus, severe and fatal asthma may be mediated by neutrophils, which is quite different from the classical Th2-driven eosinophilic form of the disease. In COPD patients, an allergic profile of inflammation can occur, particularly during exacerbation. Airway eosinophilia is observed in chronic bronchitic patients with exacerbation and is associated with the upregulation of RANTES in the airway epithelium. Recently, demonstrated that the minimization of eosinophilic airway inflammation was associated with a reduction in severe COPD exacerbation. Taken together, these studies indicate that the inflammatory characteristics of asthma and COPD are interchangeable during exacerbation and infection.

The cytokine profile is also affected by disease severity. During exacerbation, Th1/Th2 patterns are reversed to some degree in each disease. Several studies have demonstrated that the levels of IL-17, a cytokine that has been actively investigated in conditions of chronic airway inflammation such as asthma, were elevated in asthma and COPD and were correlated with the presence of neutrophils and the severity of loss of lung function. An elevated IL-17A level was associated with increased neutrophilic inflammation during severe asthma or acute exacerbation. Increased IL-17A was also correlated with increased airway hyperresponsiveness in asthmatics. These findings imply that IL-17 is an important mediator in neutrophilic asthma. In COPD, the role of IL-17 remains unclear, although the importance of IL-17 in stimulating chemokine production and the role of neutrophils and macrophages in promoting COPD pathogenesis suggest a potential connection.

Disease exacerbation in both asthma and COPD can lead to an accelerated decline in lung function. Reports have shown an association between severe asthma exacerbation and an accelerated decline in forced expiratory volume in 1 s (FEV1), to a degree similar to that seen with smoking and COPD. Another important observation was that the decline in FEV1 seen in patients with infrequent exacerbation was similar to that in a population without asthma. These findings suggest...
that repetitive episodes of exacerbation may result in fixed airflow obstruction in asthma and contribute to the phenotypic overlap between asthma and COPD.

CONVERGENCE OF ASTHMA AND COPD ON A CONTINUUM

Severe/refractory asthma: Alveolar (parenchymal) destruction

Destructive changes to the alveolar parenchyma, as in emphysema, are a representative characteristic of COPD. Emphysema is initially centrilobular, but can become panlobular in severe forms of the disease. In asthma, structural changes such as abnormal alveolar attachments and a decrease in elastic fibers can occur in the parenchyma, but these seem to be localized to the peribronchiolar spaces. These changes lead to decreased distensibility and increased collapsibility in asthmatic emphysema, whereas loss of elastic recoil is an important factor in the dynamic collapse of the airway in COPD.

Currently, it is believed that there are at least two major pathophysiological mechanisms responsible for the development of emphysema: protease–anti-protease imbalance and apoptosis of structural cells. Increased elastin degradation and enhanced expression of proteases have been documented in asthma patients. In IL-13-overexpressing transgenic mice, IL-13 induced emphysema and the expression of a variety of matrix metalloproteinases (MMPs) and cathepsins. Interventions that neutralized MMPs or cathepsins dramatically ameliorated IL-13-induced emphysematous responses. This suggests that IL-13, which is implicated in the pathogenesis of asthma, has the ability to induce an alveolar remodeling response in asthmatics. In human asthma patients, the numbers of eosinophils, CD4+ T-cells, and macrophages were increased in alveolar tissue at the time symptoms appeared. Taken together, these data indicate that the destruction of distal airways is a consequence of a chronic, long-lasting injury which can significantly affect lung function in both COPD and asthma. Furthermore, the degree of eosinophilic inflammation correlated positively with lung volume in asthma.

Asthma-like COPD: Airway remodeling

Airway remodeling is a characteristic feature of asthma and has important functional implications. The structural changes include epithelial detachment, subepithelial fibrosis, increased airway smooth muscle (ASM) mass, decreased distance between epithelium and ASM cells, goblet cell hyperplasia, mucus gland hyperplasia, proliferation of blood vessels, airway edema, and changes in cartilage. Each can contribute to airway hyperactivity (AHR) and may eventually lead to irreversible airflow obstruction with disease progression. In COPD, there is also a thickening of the airway wall, involving the epithelium, reticular basement membrane, ASM, and mucus glands, although it is not as prominent as in asthma. In addition, there is evidence for remodeling, fibrosis, and inflammation in these structures, albeit with patterns different from those seen in asthmatics. Furthermore, bronchodilator reversibility and AHR may be present in a significant proportion of COPD patients. Poorer pulmonary function is associated with a greater magnitude of AHR, and increasing severity of AHR is associated with faster rates of decline in lung function in continuing smokers. Patients with COPD who presented reversibility were often classified as having an ‘asthmatic’ component in contrast to ‘pure’ or ‘true’ COPD. Small-scale clinical trials assessing inhaled steroids in COPD indicated that they should not be prescribed for patients who had no acute response to inhaled bronchodilators or oral corticosteroids. However, many recent studies have suggested that COPD patients without AHR or bronchodilator/oral steroid responses can be treated with these drugs.

The relationship between structural alterations and airway responsiveness in COPD patients has been investigated. Chanez et al. reported that COPD patients with asthma-like features such as eosinophilia and airway hyperresponsiveness had thicker basement membranes than COPD patients without these features. There was more ASM in the small airways of COPD patients than in controls, but ASM was less prominent than in asthma. Mathematical modeling has indicated that an increase in muscle mass is the most important contributor to airway hyperresponsiveness. These findings suggest that COPD with airway remodeling is a severe phenotype and part of the overlap between COPD and asthma.

RECENT CONCEPTS FOR ASTHMA AND COPD

TREATMENT: COMMON THERAPEUTIC TARGETS

Clinical recognition of the overlap between asthma and COPD is based on inflammatory features. Inflammation in asthma is associated with increased airway hyperresponsiveness, which leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in early morning. This inflammation is present even in those with very mild asthma and is unique in that the airway wall is infiltrated by Th2 lymphocytes, eosinophils, macrophages/monocytes, and mast cells. In contrast, the pathological hallmarks of COPD are destruction of the lung parenchyma (pulmonary emphysema) and inflammation of the peripheral airways (respiratory bronchiolitis) and central airways, along with parenchymal inflammation. There is a marked increase in macrophages and neutrophils in the bronchoalveolar lavage fluid and induced sputum. Given that asthma and COPD are both pulmonary disorders characterized by various degrees of inflammation and tissue remodeling, they present common therapeutic targets.

MOLECULAR TARGETS IN ASTHMA AND COPD

Many external inflammatory signals such as viral and bacteri-
al infections, allergens, cytokines, and growth factors can activate intracellular kinases, following binding to transmembrane receptors on responsive cells. Intracellular kinase pathways play critical roles in a majority of pathobiological events, including transcription, translation, cell migration, apoptosis, and cellular production and secretion of mediators. Studies have focused on the elucidation of these signaling pathways in order to find novel therapeutic targets common to both asthma and COPD. The kinases investigated include mitogen activated protein kinases such as p38, ERK, and JNK; inhibitor of κB kinase 2/NF-κB; phosphoinositol-3 kinase; and signal-specific Janus kinases and signal transducers and activators of transcription. One inflammatory mediator common to both airway diseases is adenosine, making its receptor signaling pathway a therapeutic target for asthma and COPD. Adenosine levels were increased in the plasma, lavage fluid, and exhaled breath condensate of patients with asthma and COPD, and in animal models that exhibited features of chronic airway disease. Moreover, the inhalation of adenosine induced bronchoconstriction in patients with asthma and COPD. A non-selective adenosine receptor antagonist, theophylline, improves lung function and symptoms in asthma and COPD. Furthermore, adenosine receptors are expressed on most, if not all, inflammatory and stromal cell types involved in the pathogenesis of asthma and COPD. Extracellular adenosine elicits its effects by interacting with four adenosine receptors: A1R, A2AR, A2BR, and A3R. Adenosine receptor signaling systems are complex, displaying different and specific actions in various inflammatory responses. Studies in animal models of airway disease have suggested that A1, A3, and A2B antagonists may be useful for the treatment of asthma and COPD, although their therapeutic efficacy remains to be fully evaluated. Various selective adenosine receptor antagonists are under preclinical or clinical studies in patients with asthma and/or COPD.

CONCLUSIONS

Asthma and COPD are complex, multifactorial airway diseases associated with significant morbidity and mortality. Despite their distinct clinical phenotypic features, there is considerable overlap of symptoms and pathogenesis, and several hypotheses have been proposed regarding the status of asthma and COPD as single disease entities. Asthma and COPD may have distinct or common origins; nevertheless, clinical overlap can result owing to similar structural alterations that can develop in both diseases as a consequence of chronic inflammatory tissue injury, especially in the most severe cases. Overlapping features may occur not only in a permanent form but also as a transient symptom, as in exacerbation. In accordance with this, recent therapeutic approaches have concentrated on a target common to the pathogenesis of both asthma and COPD. Possible candidate targets include the adenosine receptor system and various kinase pathways, and many selective inhibitors of adenosine receptors and kinases are in preclinical or clinical trials. There remains a need to extend novel drug development and investigate the mechanisms and treatment of overlapping asthma and COPD.

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REFERENCES

1. Barnes PJ. Mechanisms and resistance in glucocorticoid control of inflammation. J Steroid Biochem Mol Biol 2010;120:76-85.
2. Boulet LP, Turcotte H, Hudon C, Carrier G, Maltais F. Clinical, physiological and radiological features of asthma with incomplete reversibility of airflow obstruction compared with those of COPD. Can Respir J 1998;5:270-7.
3. Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, Ligabue G, Ciaccia A, Saetta M, Papi A. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003;167:418-24.
4. Jeffery PK. Remodeling and inflammation of bronchi in asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc 2004;1:176-83.
5. Guerra S. Overlap of asthma and chronic obstructive pulmonary disease. Curr Opin Pulm Med 2005;11:7-13.
6. Gibson PG, Simpson JL. The overlap syndrome of asthma and COPD: what are its features and how important is it? Thorax 2009;64:728-35.
7. Mahler DA, Donohue JF, Barbee RA, Goldman MD, Gross NJ, Wiesniewski ME, Yancey SW, Zakes BA, Rickard KA, Anderson WH. Efficacy of salmeterol xinafoate in the treatment of COPD. Chest 1999;115:957-65.
8. Donohue JF, van Noord JA, Bateman ED, Langley SJ, Lee A, Witek TJ Jr, Kesten S, Towe L. A 6-month, placebo-controlled study comparing lung function and health status changes in COPD patients treated with tiotropium or salmeterol. Chest 2002;122:47-55.
9. Global Initiative for Asthma. Global strategy for asthma management and prevention. Bethesda: National Institutes of Health, National Heart, Lung, and Blood Institute; 2009. (NIH publication; no. 09-3659).
10. National Asthma Education and Prevention Program. Expert panel report 3: guidelines for the diagnosis and management of asthma. Bethesda: National Institutes of Health; 2007. (NIH publication; no. 07-4051).
11. Soriano JB, Davis KJ, Coleman B, Visick G, Mannino D, Pride NB. The proportional Venn diagram of obstructive lung disease: two approximations from the United States and the United Kingdom. Chest 2003;124:474-81.
12. Marsh SE, Travers J, Weatherall M, Williams MV, Aldington S, Shirtcliffe PM, Hansell AL, Nowitz MB, McNaughton AA, Soriano JB,
13. Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. Am J Respir Crit Care Med 1997;156:737-43.

14. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000;161:9-16.

15. Saetta M, Di Stefano A, Maestrelli P, Turato G, Ruggieri MP, Roggeri A, Calcagni P, Mapp CE, Ciaccia A, Fabbrì LM. Airway eosinophilia in chronic bronchitis during exacerbations. Am J Respir Crit Care Med 1994;150:1646-52.

16. Chanez P, Vignola AM, O’Shaughnessy T, Enander I, Li D, Jeffery PK, Bousquet J. Corticosteroid reversibility in COPD is related to features of asthma. Am J Respir Crit Care Med 1997;155:1529-34.

17. Ray A, Cohn L. Th2 cells and GATA-3 in asthma: new insights into the regulation of airway inflammation. J Clin Invest 1999;104:985-93.

18. Welte T, Groneberg DA. Asthma and COPD. Exp Toxicol Pathol 2006;57 Suppl 2:35-40.

19. Saetta M, Mariani M, Panina-Bordignon P, Turato G, Buonsanti C, Baraldo S, Bellettato CM, Papu A, Corbetta L, Zuin R, Sinigaglia F, Fabbrì LM. Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2002;165:1404-9.

20. Mathesons JM, Lemus R, Lange RW, Karol MH, Luster MI. Role of tumor necrosis factor in toluene diisocyanate asthma. Am J Respir Cell Mol Biol 2002;27:396-405.

21. Noguchi E, Yokouchi Y, Shibasaki M, Inoudou M, Nakahara S, Nogami T, Kamioka M, Yamakawa-Kobayashi K, Ichikawa K, Matsui A, Arimami T. Association between TNFA polymorphism and the development of asthma in the Japanese population. Am J Respir Crit Care Med 2002;166:43-6.

22. Bousquet J, Lacoste J, Chanez P, Vic P, Godard P, Michel FB. Bronchial elastic fibers in normal subjects and asthmatic patients. Am J Respir Crit Care Med 1996;153:1648-54.

23. Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. Am J Respir Cell Mol Biol 2003;28:12-24.

24. Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. Am J Respir Cell Mol Biol 2002;27:396-405.

25. James AL, Wenzel S. Clinical relevance of airway remodelling in airway disease. Thorax 2004;59:488-93.

26. Bourdin A, Serre I, Flamment H, Vic P, Neveu D, Aubas P, Godard P, Chanez P. Can endobronchial biopsy analysis be recommended to discriminate between asthma and COPD in routine practice? Thorax 2004;59:488-93.

27. Vestbo J, Prescott E. Update on the “Dutch hypothesis” for chronic respiratory disease. Thorax 1998;53 Suppl 2:S15-9.

28. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998;339:1194-200.

29. Mauad T, Dolhnikoff M. Pathologic similarities and differences between asthma and chronic obstructive pulmonary disease. Curr Opin Pulm Med 2008;14:31-8.

30. Lamblin C, Gosset P, Tille-Leblond I, Saulnier F, Marquette CH, Wallaert B, Tonnel AB. Bronchial neutrophilia in patients with noninfectious status asthmaticus. Am J Respir Crit Care Med 1998;157:394-402.
way diseases. Eur Respir J 2007;30:134-55.
48. Spurzem JR, Rennard SI. Pathogenesis of COPD. Semin Respir Crit Care Med 2005;26:142-53.
49. Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ Jr, Chapman HA Jr, Shapiro SD, Elias JA. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. J Clin Invest 2000;106:1081-93.
50. Kraft M, Djukanovic R, Wilson S, Holgate ST, Martin RJ. Alveolar tissue inflammation in asthma. Am J Respir Crit Care Med 1996;154:1505-10.
51. Kraft M, Martin RJ, Wilson S, Djukanovic R, Holgate ST. Lymphocyte and eosinophil influx into alveolar tissue in nocturnal asthma. Am J Respir Crit Care Med 1999;159:228-34.
52. Sutherland ER, Martin RJ, Bowler RP, Zhang Y, Rex MD, Kraft M. Physiologic correlates of distal lung inflammation in asthma. J Allergy Clin Immunol 2004;113:1046-50.
53. Sumi Y, Hamid Q. Airway remodeling in asthma. Allergol Int 2007;56:341-8.
54. Bosken CH, Wiggs BR, Pare PD, Hogg JC. Small airway dimensions in smokers with obstruction to airflow. Am Rev Respir Dis 1990;142:563-70.
55. Nagai A, West WW, Thurlbeck WM. The National Institutes of Health Intermittent Positive-Pressure Breathing trial: pathology studies. II. Correlation between morphologic findings, clinical findings, and evidence of expiratory air-flow obstruction. Am Rev Respir Dis 1985;132:946-53.
56. Wright JL, Lawson LM, Pare PD, Wiggs BJ, Kennedy S, Hogg JC. Morphology of peripheral airways in current smokers and ex-smokers. Am Rev Respir Dis 1983;127:474-7.
57. Kerstjens HA, Brand PL, Quanjer PH, van der Bruggen-Bogaarts BA, Koëter GH, Postma DS. Variability of bronchodilator response and effects of inhaled corticosteroid treatment in obstructive airways disease. Dutch CNSLD Study Group. Thorax 1995;48:722-9.
58. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KE, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000;356:2144-8.
59. Kumar S, Boehm J, Lee JC. p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. Nat Rev Drug Discov 2003;2:717-26.
60. Weir DC, Burge PS. Effects of high dose inhaled beclometasone dipropionate, 750 micrograms and 1500 micrograms twice daily, and 40 mg per day oral prednisolone on lung function, symptoms, and bronchial hyperresponsiveness in patients with non-asthmatic chronic airflow obstruction. Thorax 1993;48:309-16.
61. Burge PS, Calverley PM, Jones PW, Spencer S, Anderson JA, Maslen TK. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. BMJ 2000;320:1297-303.
62. Calverley PM, Burge PS, Spencer S, Anderson JA, Jones PW. Bronchodilator reversibility testing in chronic obstructive pulmonary disease. Thorax 2003;58:659-64.
63. Kuwano K, Bosken CH, Paré PD, Bai TR, Wiggs BR, Hogg JC. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. Am Rev Respir Dis 1993;148:1220-5.
64. Lambert RK, Wiggs BR, Kuwano K, Hogg JC, Paré PD. Functional significance of increased airway smooth muscle in asthma and COPD. J Appl Physiol 1993;74:2771-81.
65. Busse WW, Lemanske RF Jr. Asthma. N Engl J Med 2001;344:350-62.
66. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciruba FC, Coxson HO, Paré PD. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004;350:2645-53.