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Upregulation of neuropeptides and infant obstructive airway disorder in post-RSV wheezing and NEHI

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Abstract

Obstructive airway disorders are common in infancy and early childhood. The leading example of such disorder is post-viral wheezing, predominantly the well characterized disorder that follows respiratory syncytial virus (RSV) infection and leads to intermittent, long-term wheezing. The underlying mechanisms of the airway reactivity related to RSV infection have been extensively studies and are associated with dysregulation of the nonadrenergic-noncholinergic (NANC) system, via upregulation of neurotransmitters, typically Substance P. Neuroendocrine hyperplasia of infancy (NEHI), while a less common entity, is a disorder of infancy characterized by more severe and long-term obstructive airway disease. NEHI is pathophysiologically characterized by abundance of neuroendocrine cells in the airways containing the neuroimmune mediator bombesin, the release of which is presumed to be the driver of the persistent small airway obstruction and functional air-trapping. Here we review the NANC and NEC neurotransmitter systems and their studied roles in pulmonary diseases with a focus on their role in lung development, and subsequent various pediatric lung diseases. We focus on the juxtaposition of the separate neuroimmune mechanisms underlying the pathogenesis of post-RSV recurrent wheezing and NEHI persistent small airway obstruction. We finally raise the question whether substance P is indeed specific to post-RSV infection and bombesin to NEHI and then propose a unifying concept of post-viral spectrum of respiratory disorders that may encompass these two entities and possibly others.

Introduction

In infancy and early childhood neuropeptide released by nonadrenergic and noncholinergic (NANC) nerves and by neuroendocrine cells (NEC) are thought to play a significant pathogenetic role in lung diseases characterized by airflow limitations[1,2]. A common disorder in pediatric practice is early-childhood bronchial obstruction which, when transient and intermittent, is largely attributed to respiratory viruses[3,4] . Predominant amongst those involved in the first respiratory infection and underlying long-term wheezing is respiratory syncytial virus (RSV). The pathogenetic mechanisms inducing airway instability after RSV bronchiolitis are attributed to neuroimmune inflammatory processes, at least in part related to upregulation of the tachykinin neuropeptide substance P (Sub P) and of its receptors neurokinin (NK)1[2,5,6]. A different disorder of infancy, associated with persistent airflow limitation, is neuroendocrine hyperplasia of infancy (NEHI). This entity is classified as diffuse interstitial lung disease but is characterized physiologically by small airway obstruction, and almost never associated with structural changes or signs of inflammation [7]. NEHI also is presumed to be driven by a neuroimmune mediator, bombesin, released by pulmonary NEC [7]. During the early stage of embryonic development, NEC play a key role in growth and differentiation whilst, at the time of birth, act as airway O_2 sensors involved in neonatal adaptation to extrauterine life[3,7,8]. In infancy and early childhood, through the release of neurotransmitters, NEC are thought to be have a role in the pathogenesis of a variety of other airway disorders, including pulmonary hypertension (PH), bronchopulmonary dysplasia

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(BPD), sudden infant death syndrome (SIDS), congenital central hypoventilation syndrome (CCHS) and cystic fibrosis (CF)[9]. The aim of this manuscript is to briefly review and discuss the commonality and the differences among these entities driven by neuroimmune mechanisms, with a focus on RSV bronchiolitis and NEHI and propose a unifying paradigm between these two distinct entities.

NANC and NEC neurotransmitters

The NANC system

The complex interplay among efferent and afferent autonomic nerves in the regulation of many aspects of airway functions is beyond the scope of this paper [10]. Briefly, in addition to being regulated by the classical cholinergic bronchoconstrictor and adrenergic bronchodilator neural pathways, bronchial smooth muscle tone is also modulated by NANC nerves, which can be either inhibitory (i-NANC) or excitatory (e-NANC) (figure 1)[10,11]. Excitatory NANC-mediated bronchoconstrictor responses are believed to be under the control of a subpopulation of non-myelinated C-fibers, primary afferent neurons which release neuropeptides such as Sub P, neurokinin A and B and the calcitonin gene-related peptide. In addition to bronchoconstriction, these neurotransmitters can cause mucus secretion, bronchial artery dilatation and postcapillary venule leakiness, processes called "neurogenic inflammation" [11-13]. The excitatory NANC system is thought to play a role in allergic and nonallergic asthma, in allergen-induced bronchoconstriction and, as follows below, in the pathogenesis of RSV infection in young children[5,14].

The pulmonary NEC system

The pulmonary NEC system consists of solitary cells and distinctive clusters of these cells, termed neuroepithelial bodies (NEB), localized in the airway epithelium [3]. Pulmonary NEC express a variety of bioactive substances, including amines (serotonin and 5-HT) and neuropeptides (calcitonin gene-related peptide, gamma-aminobutyric acid, vasoactive intestinal polypeptide and bombesin). Pulmonary NEC/NEB are found in all fetal stages, at which time they are intimately involved in the regulation of lung development [3,9,15]. In fetal life, physiological hypoxia and mechanical stretch caused by fluid dynamics in the airway lumen upregulate pulmonary NEC functions, promoting epithelial cell proliferation, branching morphogenesis and type-II pneumocytes differentiation[15]. Mechanical-stretch-induced 5-HT release from NEC is mediated by mechanosensitive channels, independent of the exocytic pathway. In contrast, hypoxia-induced secretion of 5-HT and of neuropeptides occurs principally via classical exocytosis of dense core vesicles, a process mediated by voltage activated Ca^{++} channels without apparent involvement of mechanosensitive channels [3]. After birth, pulmonary NEC decrease and often disappear with lung maturation by 1-2 years of age [15]. This may not be the case in conditions of pathology, such as pediatric PH, BPD, SIDS, CCHS and CF [3,15-18]. Acute or persistent hypoxia and injury to the airways, features of some of these disorders, are recognized stimuli that can activate pulmonary NEC inducing release of bioactive neuropeptides, such as bombesin, which can modulate lung damage, as shown in premature infants with BPD, or trigger smooth muscle contraction, as shown in NEHI [3,9,15-18]. In this latter disorder, a predominant abundance of pulmonary bombesin-positive NEC in small airways and distal bronchiole has been described as the characteristic finding [18-20]. A bombesin-induced airflow limitation at that level is consistent with the clinical presentation, i.e. small airway obstruction and air-trapping[7,18].

Neuroimmune regulation of viral respiratory infection in infancy

Respiratory viruses, predominated RSV, are the leading cause of acute lower respiratory tract infections in infancy and the prime cause of hospitalization in this age population in developed countries[21,22]. Primary infection at a young age plays a pivotal role in the severity of acute disease and in subsequent recurrent wheezing, peaking in infants aged <3 months[21,23,24]. The interplay RSV-host is complex and involves cells of the innate and adaptive immune systems, whose excessive activation may induce significant cytopathic effects to the airways[24,25]. RSV infection-induced injury leads to bronchoconstriction, airway inflammation and edema. The multifaceted mechanisms provoking RSV-induced airway inflammation and hyperreactivity are still only partially understood but there is evidence that dysregulation of the NANC system is involved, favoring the bronchoconstrictive and pro-inflammatory effects of tachykinin peptides, exemplified by Sub P,

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against the bronchorelaxant effect of vasoactive intestinal peptide (VIP) [2]. RSV upregulates the expression of nerve growth factor (NGF) and of its p75 neurotrophin receptors in target cells [25]. NGF acts as promoter of acetylcholine release and as signaling molecule to induce the production of Sub P, that persists after RSV clears from the lungs[2,14,25] (figure 2A). Hence, the decreased threshold of excitatory NANC activation results from the upregulated Sub P/NK1 axis and likely underlies long-term airway dysfunction and recurrent inflammation and hyperresponsiveness [25,26]. The long-lasting sequelae of the early-life RSV infection can also be explained by the observation that the upregulated NGF also leads to short- and long-term changes in the distribution and reactivity of sensory nerves across the respiratory tract, enhancing the exaggerated functional and inflammatory reactions to infections[14,24,25]. The possible role of neuropeptides in induction of early childhood disorders characterized by recurrent wheeze in other early viral infections, such as Rhinovirus, has not been evaluated or reported.

NEC involvement in the pathogenesis of childhood disorders

As previously mentioned, abnormalities in the number, distribution and function of pulmonary NEC have been documented in a number of different pediatric lung disorders that include PH [3,18]. In normal lungs the relative density of nerve fibers increases during childhood in the arteries of the respiratory unit. In pediatric PH, a premature innervation of these arteries by nerve fibers occurs and associated with release of vasoconstrictor peptides during the first year of life[15,27]. Pulmonary NEC may be involved in the pathophysiology of PH through the production and release of 5-hydroxytryptamine 5-HT, a potent vasoconstrictor, whose release is amplified by hypoxia [3,16]. PNEC are situated in small peripheral airways and at bronchoalveolar portals, in close proximity to pulmonary arterioles that are involved in hypoxia induced vascular resistance [3]. To date there are no studies of pulmonary NEC in pediatric PH, however, immunohistochemical studies of lungs from adult patients with PH, both primary and secondary to congenital heart disease, revealed significant hyperplasia of these cells in early stages of the disease [28]. Significant hyperplasia of pulmonary NEC has been described in infants with SIDS and CCHS, disorders characterized by dysfunction of respiratory control, and in the "pre-surfactant era" BPD, possibly related to chronic hypoxia and/or release of mitogenic inflammatory cytokines release of inflammatory cytokines that could stimulate the mitogenesis of PNEC/NEB directly or enhance recruitment from precursor cells[3,15,29,30]. The increased release of bombesin-like peptides (BLP) by pulmonary NEC in BPD has been attributed to the lung inflammatory response observed in this disorder but also questioned as a response to lung injury[3,31,32]. Increased levels of BLP are detectable in urine of infants with BPD that exhibit a variety of physiological abnormalities, including pulmonary hypertension, airway hyperreactivity, and increased apneic spells [33]. Finally, in CF reduction in neuropeptides secretion by CFTR-deficient NEC could exacerbate the disease process by negatively affecting composition of periciliary fluid, and eventually leading to airway plugging and obstruction [3,34]. A recent study on lung tissues from $CFTR^{-/-}$ deficient mice, showed altered distribution and frequency of pulmonary NEC/NEB, abnormal innervation with reduced airway size during different developmental stages, suggesting an intrinsic abnormality [17]. Through potentiating cholinergic neurotransmission, neuropeptides can act on bronchial smooth muscle, mucosal vasculature and submucosal glands and induce airflow obstruction and by promoting recruitment and activation of granulocytes, exacerbate neurogenic inflammation[35,36]. Pulmonary NEC may play a proinflammatory role via production of neuropeptides in these pathologies and possibly, as will be reviewed in the next two paragraphs, in respiratory virus-induced airway diseases. These latter disorders are characterized by inflammation, release of oxygen radicals, injury to airway structures, and, clinically by acute hypoxia, all stimuli that can activate pulmonary NEC causing release of bioactive neuropeptides[16-18,25,30].

NEHI

Since first described, NEHI has gained its place as a distinct entity amongst rare causes of infantile diffuse lung disease[37]. NEHI clinically presents with persistent tachypnea, retractions, crackles, and hypoxemia and physiologically as small airway obstruction evidenced by reduction of FEF_{75}, FEF_{85}, and FVC[38-40]. Symptoms are not detectable at birth but usually present before 12 months (mean: 4 months, interquartile range = 2-6 months) [41]. Remarkable in their absence are prematurity, evidence of pulmonary dysmatura-

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tion, underlying causes of diffuse lung disease, congenital heart disease, or genetic characteristics [3,39,40,42,43]. However, a recent review on 117 NEHI children demonstrated that 17% of them had evidence of immune system abnormalities including low immunoglobulin (Ig)G and IgA levels, low complement 3 concentrations, and cyclic neutropenia of infancy[41]. Steroids and bronchodilators show no effect in NEHI and the sole effective element of supportive care is supplemental oxygen. While resolution is predictable and spontaneous, disease duration often lasts years [39,44]. Chest x-ray findings are non-specific. However, high-resolution CT scans display characteristic findings of ground glass opacities, involving more than one lobe, and air-trapping. These changes are deemed diagnostic and have largely replaced lung biopsy for the diagnosis[40,42,44,45]. Bronchoscopy reveals no structural changes or inflammation. Lung biopsy, the standard diagnostic method for diffuse interstitial lung diseases, shows a paucity of airway or parenchyma abnormalities, no inflammation, but characteristically, increased bombesin positive NEC cells in bronchioles and alveoli[3,18,39,40,42,44]. The positive correlation between pulmonary bombesin-positive NEC density and small airway obstruction severity suggests that bombesin plays a causative role in the pathophysiology of NEHI [39,40]. Finally, bronchoalveolar lavage in NEHI revealed low white blood cell counts and decreased inflammatory markers, paralleling lung biopsy findings of sparse inflammation[46]. Hence, despite the positive correlation between the load of pulmonary NEC and severity of obstruction, a discrepancy remains between abundant pulmonary NEC and paucity of inflammation and histological abnormalities, as compared to BPD and other conditions with increased pulmonary NEC. An alternative explanation for NEHI proposed by recent publications, suggests that pulmonary neuroendocrine cells are a marker of airway underdevelopment and immaturity [18,19,43] and persistence of bombesin is shared in postnatal life by a variety of infantile pathologies.[18] Due to low prevalence and lack of animal models, the etiology and pathophysiology of NEHI remain elusive.

The post-viral infection disorder spectrum speculation.

A striking pathophysiological commonality between post-RSV pulmonary dysfunction and NEHI is the upregulation of neurotransmitters. As described above, during and after RSV lower respiratory tract infection airway hyperresponsiveness, increased vascular permeability and neurogenic inflammation are largely attributed to substance P and its upregulated NK1 receptor (figure 2A). In NEHI, bombesin is thought to be involved in the pathogenesis of small airway obstruction but may also play a protective role against noxious agents, such as respiratory viruses (figure 2B and table 1). The hypothesis that viral infections might underlie NEHI is supported by Gomes et al., reporting viral infection preceding the onset of clinical symptoms in all infants in a large NEHI case series [44]. While both bombesin and substance P are neurotransmitters, and intuitively could have similar functional roles, bombesin is viewed as the marker of NEHI while Sub P has not been studied in NEHI. The reverse has been the case for post-RSV wheezing models, where substance P has been the extensively studied neurokinin and bombesin has not. In these and other disorders characterized by airflow limitation it would therefore be interesting to evaluate a pathogenetic role of both these neurokinins, as well as other "bronchoconstrictor" neuropeptides, such as the calcitonin gene-related peptide that is known to be released by both NENC fibers and NEC[47,48]. Combined effects of substance P and bombesin were found in pathologies such as rheumatoid arthritis and histamine-independent itch and both neurokinins are secreted by neurons in other organ systems[49-51]. Both these respiratory entities have airway obstruction as their main clinical presentation. This is more severe and long term in NEHI patients, and often associated with persistent hypoxemia, but with symptoms improving over time, although non-atopic asthma may develop in the follow-up[42]. While the presentation is milder in post-RSV wheezing, the affected children have increased risk of bronchial hyperreactivity and asthma persisting into older ages[52,53]. Physiologically, pulmonary function tests in post- RSV wheezing and NEHI showed similar obstructive pattern (Table 1), except for consistent absent response to ?$_2$-agonists in NEHI [20,42,52,54]. Radiologic studies in NEHI, in addition to the ground-glass opacification predominantly involving the right middle lobe and lingula, indicate a mix of hyperinflation with collapsed areas, pointing towards small airway obstruction in line with the clinical and PFT changes [38,45]. These observations for NEHI suggest functional air-trapping, i.e., increased tone of airway smooth muscle, much like that described in airway hyperreactivity post-RSV, but with the distinction of being persistent, not intermittent and irreversible by bronchodilators. Fluctuation of symptom severity over time in NEHI and patchy radiological distribution further point to functional *vs*

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. structural pathogenesis of NEHI. These data suggest that increased neurokinin activity is involved in the pathophysiology of both conditions, with persistence thereof in NEHI, and oscillations in post-RSV wheezing. Whether these differences veritably reflect a biological difference where the contribution of different cells and the relative abundance of the two neurokinins, and possibly involvement of other neurotransmitters, might play different roles in determining the characteristics of one entity or the other, *vs.* having been serendipitously differentially researched in the two entities, is a matter of speculation. Genetic mutations may be involved in the pathogenesis of both disorders. While in the cases of post-RSV wheezing, a "familial" presentation may go unnoticed, or interpreted as being a marker of familial asthma, in NEHI, due to the rarity of the disease, its severity and long-term morbidity, the condition may be clearly identified when it affects multiple family members. NEHI familial cases are described and a heterozygous *NKX2.1* mutation has been identified in an infant with classic presentation of NEHI and in four other adult family members with histories of childhood lung disease [55,56]. This mutation strongly segregated with lung disease in this family but not in eight others unrelated NEHI subjects, suggesting that altered expression of *NKX2.1* target gene may be involved in pathophysiology of NEHI, but is not the predominant cause of the disease[56]. Therefore, one may speculate that subjects with genetically determined or acquired autonomic pathway defects may have a more profound response to a second hit (e.g., viral infection), as has been shown for the predictable tendency to wheeze following rhinovirus infections based on genetics[57]. The recent observation that a significant proportion of NEHI children may have evidence of immune system abnormalities support the notion that defects in the immune response to infection might be involved [41].

These observations may point to a continuum of airway disease determined by an innate or acquired tendency to respond to some hits (likely viruses) with a disproportionate response. In this context, that in post-viral obstructive disease the severity of the response might be determined by an underlying genetic abnormality. Alternatively, if one accepts the notion that the presence of bombesin is a marker of dysmaturity [18,19,43] the fact that the presentation of NEHI is delayed beyond the neonatal period, points to the need for a second hit for a clinical presentation to emerge. It is tempting to expand the hypothesis to include post-infectious bronchiolitis obliterans (PIBO), a disorder where viral infection leads to severe structural airway damage[58]. While discussion on PIBO has focused on adenovirus, recent literature expands the spectrum of underlying organisms to include RSV as a possible trigger[58]. Neurokinins have not been studied in PIBO during the acute or chronic phases, but along the lines of the discussion on "familial" cases of NEHI, some individuals respond to adenoviral infections and develop PIBO, while most do not. An Argentinean study identified children with PIBO as having mannose-binding lectin insufficiency, supporting the notion that a genetic abnormality may determine severity of the response[59]. This innate tendency could be the unifying factor that determines at which level on the spectrum - from wheezing to NEHI to PIBO - will individuals end post viral infection.

Conclusion.

In infancy and early childhood neuropeptide released by nonadrenergic and noncholinergic (NANC) nerves and by neuroendocrine cells (NEC) play a significant pathogenetic role in a wide variety of lung diseases. An interesting observation is the different sequelae driven by two neuroimmune mediators, substance P and bombesin, respectively in post-RSV wheezing and NEHI. It would be interesting to explore whether these respective mediators are upregulated in both conditions, rather than specific to one or the other. The finding of long-term persistence of RSV in bone marrow may indicate that while absent in the lung, the virus' long-term sequelae may be governed by persistent extrapulmonary immune activity. If one accepts that NEHI may be a sequel of RSV or other viral infection, while cognizant of the limitations imposed by absence of animal models of NEHI, it would be of interest to explore virus permanence in bone marrow of such patients. Finally, if persistent neurokinin activity constitutes the underlying factor determining some of these morbidities, blockers of these molecules may offer future specific therapeutic targets.

References

1. Barnes PJ. Neuropeptides in human airways: function and clinical implications. *Am Rev Respir Dis.* 1987;136(6 Pt 2):S77-83.

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2. Piedimonte G. Neural mechanisms of respiratory syncytial virus-induced inflammation and prevention of respiratory syncytial virus sequelae. *Am J Respir Crit Care Med.* 2001;163(3 Pt 2):S18-21.

3. Cutz E, Yeger H, Pan J. Pulmonary neuroendocrine cell system in pediatric lung disease-recent advances. *Pediatr Dev Pathol.*2007;10(6):419-435.

4. Le Souef P. Viral infections in wheezing disorders. *Eur Respir Rev.* 2018;27(147).

5. Rossi GA, Colin AA. Infantile respiratory syncytial virus and human rhinovirus infections: respective role in inception and persistence of wheezing. *Eur Respir J.* 2015;45(3):774-789.

6. Jartti T, Gern JE. Role of viral infections in the development and exacerbation of asthma in children. *J Allergy Clin Immunol.*2017;140(4):895-906.

7. Carr LL, Kern JA, Deutsch GH. Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia and Neuroendocrine Hyperplasia of Infancy. *Clin Chest Med.* 2016;37(3):579-587.

8. Aguayo SM, Schuyler WE, Murtagh JJ, Jr., Roman J. Regulation of lung branching morphogenesis by bombesin-like peptides and neutral endopeptidase. *Am J Respir Cell Mol Biol.* 1994;10(6):635-642.

9. Johnson DE, Georgieff MK. Pulmonary neuroendocrine cells. Their secretory products and their potential roles in health and chronic lung disease in infancy. *Am Rev Respir Dis.* 1989;140(6):1807-1812.

10. van der Velden VH, Hulsmann AR. Autonomic innervation of human airways: structure, function, and pathophysiology in asthma.*Neuroimmunomodulation.* 1999;6(3):145-159.

11. Stretton D. Non-adrenergic, non-cholinergic neural control of the airways. *Clin Exp Pharmacol Physiol.* 1991;18(10):675-684.

12. de Jongste JC, Jongejan RC, Kerrebijn KF. Control of airway caliber by autonomic nerves in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis.* 1991;143(6):1421-1426.

13. Douglas SD, Leeman SE. Neurokinin-1 receptor: functional significance in the immune system in reference to selected infections and inflammation. *Ann N Y Acad Sci.* 2011;1217:83-95.

14. Piedimonte G, Rodriguez MM, King KA, McLean S, Jiang X. Respiratory syncytial virus upregulates expression of the substance P receptor in rat lungs. *Am J Physiol.* 1999;277(4):L831-840.

15. Cutz E. Hyperplasia of pulmonary neuroendocrine cells in infancy and childhood. *Semin Diagn Pathol.* 2015;32(6):420-437.

16. Morecroft I, Heeley RP, Prentice HM, Kirk A, MacLean MR. 5-hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: importance of the 5-HT1B receptor. *Br J Pharmacol.* 1999;128(3):730-734.

17. Pan J, Luk C, Kent G, Cutz E, Yeger H. Pulmonary neuroendocrine cells, airway innervation, and smooth muscle are altered in Cftr null mice. *Am J Respir Cell Mol Biol.* 2006;35(3):320-326.

18. Emiralioglu N, Orhan D, Cinel G, et al. Variation in the bombesin staining of pulmonary neuroendocrine cells in pediatric pulmonary disorders-A useful marker for airway maturity. *Pediatr Pulmonol.*2020.

19. Yancheva SG, Velani A, Rice A, et al. Bombesin staining in neuroendocrine cell hyperplasia of infancy (NEHI) and other childhood interstitial lung diseases (chILD). *Histopathology.*2015;67(4):501-508.

20. Young LR, Brody AS, Inge TH, et al. Neuroendocrine cell distribution and frequency distinguish neuroendocrine cell hyperplasia of infancy from other pulmonary disorders. *Chest.* 2011;139(5):1060-1071.

21. Simoes EA. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. *J Pediatr.* 2003;143(5 Suppl):S118-126.

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22. Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. *N Engl J Med.*2015;372(9):835-845.

23. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child.*1986;140(6):543-546.

24. Rossi GA, Medici MC, Arcangeletti MC, et al. Risk factors for severe RSV-induced lower respiratory tract infection over four consecutive epidemics. *Eur J Pediatr.* 2007;166(12):1267-1272.

25. Rossi GA, Colin AA. Respiratory syncytial virus-Host interaction in the pathogenesis of bronchiolitis and its impact on respiratory morbidity in later life. *Pediatr Allergy Immunol.*2017;28(4):320-331.

26. Piedimonte G. Contribution of neuroimmune mechanisms to airway inflammation and remodeling during and after respiratory syncytial virus infection. *Pediatr Infect Dis J.* 2003;22(2 Suppl):S66-74; discussion S74-65.

27. Allen KM, Wharton J, Polak JM, Haworth SG. A study of nerves containing peptides in the pulmonary vasculature of healthy infants and children and of those with pulmonary hypertension. *Br Heart J.*1989;62(5):353-360.

28. Heath D, Yacoub M, Gosney JR, Madden B, Caslin AW, Smith P. Pulmonary endocrine cells in hypertensive pulmonary vascular disease.*Histopathology.* 1990;16(1):21-28.

29. Gillan JE, Cutz E. Abnormal pulmonary bombesin immunoreactive cells in Wilson-Mikity syndrome (pulmonary dysmaturity) and bronchopulmonary dysplasia. *Pediatr Pathol.* 1993;13(2):165-180.

30. Perrin DG, McDonald TJ, Cutz E. Hyperplasia of bombesin-immunoreactive pulmonary neuroendocrine cells and neuroepithelial bodies in sudden infant death syndrome. *Pediatr Pathol.* 1991;11(3):431-447.

31. Gonzalez N, Moody TW, Igarashi H, Ito T, Jensen RT. Bombesin-related peptides and their receptors: recent advances in their role in physiology and disease states. *Curr Opin Endocrinol Diabetes Obes.* 2008;15(1):58-64.

32. Sunday ME, Shan L, Subramaniam M. Immunomodulatory functions of the diffuse neuroendocrine system: implications for bronchopulmonary dysplasia. *Endocr Pathol.* 2004;15(2):91-106.

33. Cullen A, Van Marter LJ, Allred EN, Moore M, Parad RB, Sunday ME. Urine bombesin-like peptide elevation precedes clinical evidence of bronchopulmonary dysplasia. *Am J Respir Crit Care Med.*2002;165(8):1093-1097.

34. Pan J, Bear C, Farragher S, Cutz E, Yeger H. Cystic fibrosis transmembrane conductance regulator modulates neurosecretory function in pulmonary neuroendocrine cell-related tumor cell line models. *Am J Respir Cell Mol Biol.* 2002;27(5):553-560.

35. Belvisi MG, Stretton CD, Barnes PJ. Bombesin-induced bronchoconstriction in the guinea pig: mode of action. *J Pharmacol Exp Ther.* 1991;258(1):36-41.

36. Solway J, Leff AR. Sensory neuropeptides and airway function.*J Appl Physiol (1985).* 1991;71(6):2077-2087.

37. Kurland G, Deterding RR, Hagood JS, et al. An official American Thoracic Society clinical practice guideline: classification, evaluation, and management of childhood interstitial lung disease in infancy. *Am J Respir Crit Care Med.* 2013;188(3):376-394.

38. Brody AS, Crotty EJ. Neuroendocrine cell hyperplasia of infancy (NEHI). *Pediatr Radiol.* 2006;36(12):1328.

39. Deterding RR, Fan LL, Morton R, Hay TC, Langston C. Persistent tachypnea of infancy (PTI)–a new entity. *Pediatr Pulmonol.*2001;Suppl 23:72-73.

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40. Deterding RR, Pye C, Fan LL, Langston C. Persistent tachypnea of infancy is associated with neuroendocrine cell hyperplasia.*Pediatr Pulmonol.* 2005;40(2):157-165.

41. Liptzin DR, Pickett K, Brinton JT, et al. Neuroendocrine Cell Hyperplasia of Infancy. Clinical Score and Comorbidities. *Ann Am Thorac Soc.* 2020;17(6):724-728.

42. Lukkarinen H, Pelkonen A, Lohi J, et al. Neuroendocrine cell hyperplasia of infancy: a prospective follow-up of nine children.*Arch Dis Child.* 2013;98(2):141-144.

43. Bush A, Griese M, Seidl E, Kerem E, Reu S, Nicholson AG. Early onset children's interstitial lung diseases: Discrete entities or manifestations of pulmonary dysmaturity? *Paediatr Respir Rev.*2019;30:65-71.

44. Gomes VC, Silva MC, Maia Filho JH, et al. Diagnostic criteria and follow-up in neuroendocrine cell hyperplasia of infancy: a case series.*J Bras Pneumol.* 2013;39(5):569-578.

45. Brody AS, Guillerman RP, Hay TC, et al. Neuroendocrine cell hyperplasia of infancy: diagnosis with high-resolution CT. *AJR Am J Roentgenol.* 2010;194(1):238-244.

46. Popler J, Wagner BD, Tarro HL, Accurso FJ, Deterding RR. Bronchoalveolar lavage fluid cytokine profiles in neuroendocrine cell hyperplasia of infancy and follicular bronchiolitis. *Orphanet J Rare Dis.* 2013;8:175.

47. Palmer JB, Cuss FM, Mulderry PK, et al. Calcitonin gene-related peptide is localised to human airway nerves and potently constricts human airway smooth muscle. *Br J Pharmacol.* 1987;91(1):95-101.

48. Shimosegawa T, Said SI. Co-occurrence of immunoreactive calcitonin and calcitonin gene-related peptide in neuroendocrine cells of rat lungs. *Cell Tissue Res.* 1991;264(3):555-561.

49. Akiyama T, Tominaga M, Takamori K, Carstens MI, Carstens E. Roles of glutamate, substance P, and gastrin-releasing peptide as spinal neurotransmitters of histaminergic and nonhistaminergic itch.*Pain.* 2014;155(1):80-92.

50. Grimsholm O, Guo Y, Ny T, Rantapaa-Dahlqvist S, Forsgren S. Are neuropeptides important in arthritis? Studies on the importance of bombesin/GRP and substance P in a murine arthritis model. *Ann N Y Acad Sci.* 2007;1110:525-538.

51. Panula P, Hadjiconstantinou M, Yang HY, Costa E. Immunohistochemical localization of bombesin/gastrin-releasing peptide and substance P in primary sensory neurons. *J Neurosci.* 1983;3(10):2021-2029.

52. Sigurs N, Aljassim F, Kjellman B, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life.*Thorax.* 2010;65(12):1045-1052.

53. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years.*Lancet.* 1999;354(9178):541-545.

54. Kerby GS, Wagner BD, Popler J, et al. Abnormal infant pulmonary function in young children with neuroendocrine cell hyperplasia of infancy. *Pediatr Pulmonol.* 2013;48(10):1008-1015.

55. Popler J, Gower WA, Mogayzel PJ, Jr., et al. Familial neuroendocrine cell hyperplasia of infancy. *Pediatr Pulmonol.*2010;45(8):749-755.

56. Young LR, Deutsch GH, Bokulic RE, Brody AS, Nogee LM. A mutation in TTF1/NKX2.1 is associated with familial neuroendocrine cell hyperplasia of infancy. *Chest.* 2013;144(4):1199-1206.

57. Caliskan M, Bochkov YA, Kreiner-Moller E, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med.*2013;368(15):1398-1407.

58. Li YN, Liu L, Qiao HM, Cheng H, Cheng HJ. Post-infectious bronchiolitis obliterans in children: a review of 42 cases. *BMC Pediatr.* 2014;14:238.

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59. Giubergia V, Salim M, Fraga J, et al. Post-infectious bronchiolitis obliterans and mannose-binding lectin insufficiency in Argentinean children. *Respirology.* 2015;20(6):982-986.

Legends for Figures.

Figure 1. Bronchial smooth muscle tone is regulated by cholinergic bronchoconstrictor and the adrenergic bronchodilator neural pathways, but also by NANC nerves, which can be either inhibitory (i-NANC) or excitatory (e-NANC). E-NANC-mediated bronchoconstriction is under the control of a subpopulation of non-myelinated C-fiber primary afferent neurons which release Sub P. Bronchial smooth muscle tone is also increased by the pulmonary NEC cells, system localized in the airway epithelium that express a variety of bioactive substances including the neuropeptides bombesin. Vasoactive intestinal peptide (VIP) counteracts the bronchoconstrictive effect of Sub P and bombesin the bronchorelaxant, whilst norepinephrine (NE) inhibits the release of acetylcholine by the vagus nerve.

Figure 2. A. During and after RSV lower respiratory tract infection airway hyperresponsiveness, increased vascular permeability and neurogenic inflammation are largely attributed to substance P and its upregulated NK1 receptor. B. In NEHI, bombesin is thought to be involved in the pathogenesis of small airway obstruction, but is may also play a protective role against noxious agents, such as respiratory viruses.

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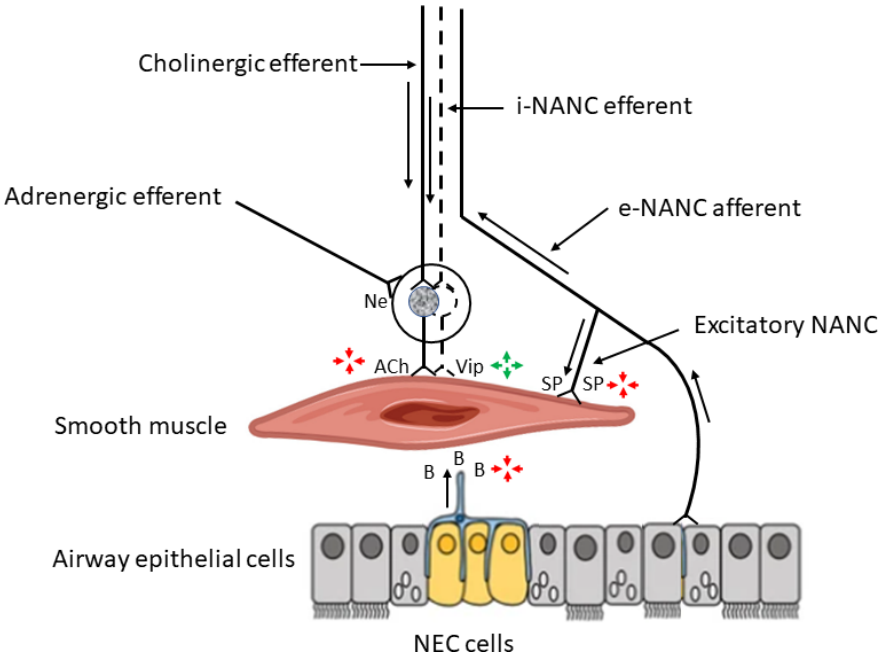

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