CHEST

Is Alveolar Macrophage Phagocytic Dysfunction in Children With Protracted Bacterial Bronchitis a Forerunner to Bronchiectasis?

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BACKGROUND: Children with recurrent protracted bacterial bronchitis (PBB) and bronchiectasis share common features, and PBB is likely a forerunner to bronchiectasis. Both diseases are associated with neutrophilic inflammation and frequent isolation of potentially pathogenic microorganisms, including nontypeable *Haemophilus influenzae* (NTHi), from the lower airway. Defective alveolar macrophage phagocytosis of apoptotic bronchial epithelial cells (efferocytosis), as found in other chronic lung diseases, may also contribute to tissue damage and neutrophil persistence. Thus, in children with bronchiectasis or PBB and in control subjects, we quantified the phagocytosis of airway apoptotic cells and NTHi by alveolar macrophages and related the phagocytic capacity to clinical and airway inflammation.

METHODS: Children with bronchiectasis (n = 55) or PBB (n = 13) and control subjects (n = 13) were recruited. Alveolar macrophage phagocytosis, efferocytosis, and expression of phagocytic scavenger receptors were assessed by flow cytometry. Bronchoalveolar lavage fluid interleukin (IL) 1β was measured by enzyme-linked immunosorbent assay.

RESULTS: For children with PBB or bronchiectasis, macrophage phagocytic capacity was significantly lower than for control subjects ($P = .003$ and $P < .001$ for efferocytosis and $P = .041$ and $P = .004$ for phagocytosis of NTHi; PBB and bronchiectasis, respectively); median phagocytosis of NTHi for the groups was as follows: bronchiectasis, 13.7% (interquartile range [IQR], 11%-16%); PBB, 16% (IQR, 11%-16%); control subjects, 19.0% (IQR, 13%-21%); and median efferocytosis for the groups was as follows: bronchiectasis, 14.1% (IQR, 10%-16%); PBB, 16.2% (IQR, 14%-17%); control subjects, 18.1% (IQR, 16%-21%). Mannose receptor expression was significantly reduced in the bronchiectasis group ($P = .019$), and IL-1β increased in both bronchiectasis and PBB groups vs control subjects.

CONCLUSIONS: A reduced alveolar macrophage phagocytic host response to apoptotic cells or NTHi may contribute to neutrophilic inflammation and NTHi colonization in both PBB and bronchiectasis. Whether this mechanism also contributes to the progression of PBB to bronchiectasis remains unknown. CHEST 2016; 149(2):508-515

KEY WORDS: bronchiectasis; childhood lung disease; inflammation; macrophage; phagocytosis; protracted bacterial bronchitis

ABBREVIATIONS: BALF = bronchoalveolar lavage fluid; CF = cystic fibrosis; IL = interleukin; MR = mannose receptor; NTHi = nontypeable *Haemophilus influenzae*; PBB = protracted bacterial bronchitis

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Bronchiectasis remains a major contributor to chronic respiratory morbidity and mortality.[1-4] Poor or inadequate treatment leads to loss of lung function[5] and subsequent reduction in life expectancy.[6] The host response to infection of children with postinfectious or idiopathic bronchiectasis has rarely been studied and is a potential contributory factor to the development of bronchiectasis.

Protracted bacterial bronchitis (PBB) is a relatively new and significant diagnostic clinical entity first described by our group in Australia.[7] PBB is important because it is a common cause of chronic wet cough in children, evident through its incorporation in national[8] and international cough guidelines.[9] Given the link (eg, clinical symptoms, microbiome) between PBB, recurrent PBB, and bronchiectasis,[10,11] a better understanding of the pathobiologic factors that underlie both diseases would be clinically useful.

We have described a marked airway neutrophilia in children with bronchiectasis and PBB.[7] Neutrophilic airway inflammation and its persistence occur by a variety of mechanisms. We and others have identified an abnormal accumulation of apoptotic bronchial epithelial cells in the airways of adults with chronic inflammatory lung diseases, including COPD, noneosinophilic asthma, and bronchiectasis.[12-14] We then showed that apoptotic neutrophils and epithelial cells were inefficiently phagocytosed (a process termed *efferocytosis*) by alveolar macrophages in these chronic lung diseases.[15-18] The uncleared material could undergo secondary necrosis, potentially contributing to a perpetuation of chronic inflammation leading to episodic respiratory symptoms, infection, and tissue damage.[12,19] In adults with cystic fibrosis (CF) and non-CF bronchiectasis, impaired phagocytosis was related to byproducts of neutrophils.[20] Several macrophage-targeted therapies were shown to improve alveolar macrophage phagocytic function[17,21,22]; therefore, elucidating whether the defective phagocytosis or efferocytosis function is present in PBB and bronchiectasis is important to better understand the pathobiology of these diseases in children and to inform future therapeutics. To our knowledge there are no published studies on efferocytosis in children's airway macrophages.

Nontypeable *Haemophilus influenzae* (NTHi) is the most common pathogen identified in children with bronchiectasis and PBB, being present at clinically important levels in bronchoalveolar lavage fluid (BALF) in almost 50% of children with bronchiectasis.[23,24] It is thus biologically plausible that in addition to a reduced ability to phagocytose apoptotic cells, alveolar macrophages in bronchiectasis and PBB have a reduced ability to phagocytose NTHi. This may facilitate bacterial persistence and biofilm formation in the conducting airways and may be involved in the progression of PBB to bronchiectasis.

Our primary aim was to quantify phagocytosis of airway apoptotic cells and NTHi by alveolar macrophages from children with bronchiectasis and PBB, as well as from control children. Our secondary aims were to determine whether phagocytic capacity was associated with clinical or demographic variables and to determine differing patterns of airway inflammation. Given our previous finding that interleukin (IL) 1β and related mediators were associated with BALF neutrophils, cough symptoms, and disease recurrence,[25] we included IL-1β as a key inflammatory marker.

Materials and Methods

Subjects

Children younger than 10 years old who underwent flexible bronchoscopy at two Australian centers (Darwin and Brisbane) were eligible for inclusion. Bronchoscopy for children with bronchiectasis and PBB has been standard clinical practice in our setting for years in accordance to local and international guidelines.[8,9,26] Upon informed consent, a standardized medical history was taken (focus on respiratory history including cough quality [wet vs dry]).[27] Groups were (1) bronchiectasis (n = 55), defined by pediatric chest high-resolution CT criteria[28]; and (2) PBB (n = 13), children with clinical features of PBB,[8] ie, isolated chronic (>4 weeks) wet cough. They then received treatment with prolonged antibiotics and were deemed to have PBB if their cough responded to antibiotics within 2 weeks[8,11]; and (3) control subjects (n = 13), children undergoing bronchoscopy for other reasons, for example, for evaluation of stridor or reevaluation of foreign body inhalation. Exclusion criteria

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