neutrophilic infiltration in the lungs (Fig. 7, *A* and *B*). Furthermore, the protein level of Muc5ac in the BAL fluid, as evaluated by ELISA, was significantly elevated by the enforced expression of pendrin (Fig. 7*C*), which is associated with enhanced airway hyperreactivity (Fig. 7*D*), and with hyperproduction of Cxcl1 (KC) and Cxcl2 (MIP2), mouse chemoattractants for neutrophils, in BAL fluid (Fig. 7*E*). These results provide formal proof that pendrin overexpression can induce neutrophilic inflammation and mucus production in BECs in vivo.

Discussion

Mucus overproduction is a hallmark of bronchial asthma and is closely related to morbidity and mortality of this disease (2). However, the underlying mechanism of this process is still poorly understood. Previous studies have shown that IL-13 is critical in mucus overproduction in bronchial asthma (7–9). In the present study, based on the findings that anion transporters play an important role in mucus production (11), we found pendrin to be a novel IL-13/IL-4-inducible molecule that is a multispanning transmembrane protein and acts as an anion transporter (Figs. 2, 3, and 5 and data not shown). OVA inhalation in mice caused asthmatic phenotypes, including mucus overproduction that was accompanied by increased pendrin expression at the apical surface of BECs (Fig. 3). Kuperman et al. previously showed that the *Pds* gene was included in all gene profiles of three different asthmatic models: wild-type mice exposed to OVA, mice expressing IL-13 in the epithelium, and mice expressing IL-13 in epithelium with restricted STAT6 expression in nonciliated airway epithelial cells (39). Likewise, Pedemonte et al. recently demonstrated that IL-4 induced pendrin expression in human BECs and that pendrin regulated thiocyanate transport that is essential for innate defense in bronchial mucosa (40). These findings are therefore consistent with our current finding that pendrin is a novel IL-13/IL-4-inducible gene in airway epithelial cells. However, no apparent abnormal lung phenotype has been reported in *Pds* knockout mice (19), and the functional role of pendrin in mucus production in bronchial asthma has not been addressed.

To formally test whether pendrin could directly regulate mucus production in BECs, we generated pendrin-expressing NCI-H292 cells in vitro. We found that pendrin functioned as an efficient anion transporter and remarkably induced the gene and protein expression of MUC5AC, a major mucus protein in asthma and COPD patients (Fig. 5). However, the precise molecular mechanism of mucus gene induction by pendrin is still unclear. *MUC5AC* has several putative *cis*-elements of transcription factors such as NF-κB, Sp1, and AP1 within its promoter sequence (4). Thus, one possibility is that pendrin somehow directly activates these transcription factors to induce mucus gene expression. In contrast, another possibility is that pendrin induces several inflammatory mediators such as TGF-α, EGF, and TNF-α, which regulate the transcription of the *MUC5AC* gene via activation of the transcription factors, as mentioned above (4). We showed that the anion transport activity of pendrin was strictly correlated with its mucus production (Fig. 6). Thus, aberrant anion transport activities by pendrin in BECs may activate either pathway, inducing the expression of mucin genes. Further work is underway to test which possibility is the case in our system.

To further elucidate the role of pendrin in vivo, we forced the expression of pendrin in mouse lungs and found mucus overproduction in airway tracts accompanied by neutrophil-dominant inflammation (Fig. 7). In this system, mucus production may be induced not only by a direct effect of pendrin on airway epithelial cells, but also by an indirect effect of pendrin by recruiting inflammatory cells, particularly neutrophils, as occurred in COPD. The precise mechanism whereby neutrophils are predominantly recruited into the lungs remains to be addressed. Based on our finding that Cxcl1 (KC) and Cxcl2 (MIP2) production were detected in BAL fluid, pendrin may directly or indirectly trigger the production of these chemoattractants toward neutrophils. Neutrophil elastase released from infiltrated neutrophils in the lungs enhances expression of mucin genes by both transcriptional and posttranscriptional mechanisms (4): neutrophil elastase activates the EGFR signals via several inflammatory mediators, including TGF-α, followed by activation of the MAP kinase pathway and the aforementioned transcription factors, thus leading to up-regulation of *MUC5AC* or *MUC2* genes; and neutrophil elastase prolongs the half-life of the MUC5AC mRNA. However, to firmly establish the contribution of pendrin in the pathogenesis of bronchial asthma and COPD, additional studies are needed that target patients and that use pendrin in knock-out mice.

Generally, infiltration of eosinophils, rather than neutrophils, is a typical feature of bronchial asthma. In severely exacerbated asthma, however, infiltration of neutrophils appears to be noted (1). It is often thought that neutrophil infiltration in asthmatic lungs is due to extrinsic factors other than allergens, such as viruses, LPS, and ozone (41); however, an allergen itself, such as OVA, could induce infiltration of neutrophils (42). Indeed, the profile of BAL cells and lung tissues in OVA-induced asthmatic mice exhibited a transition from neutrophil- to eosinophil-dominant inflammation in a time-dependent manner (H. Matsushita et al., unpublished data). These results clearly suggest that neutrophil infiltration is a cardinal feature of bronchial asthma itself in its early stage, after allergen exposure. Taken together, pendrin induced by IL-13/IL-4 signals could cause mucus overproduction dependent upon neutrophil inflammation.

Mucus overproduction is also a prominent feature of COPD along with metaplasia of submucosal glands and goblet cells, and MUC5AC protein expression is significantly higher in the bronchiolar epithelium of patients with COPD (3). In COPD, there is excessive activity of proteases as well as an imbalance between proteases and endogenous antiproteases. In particular, the aforementioned neutrophil elastase is a major constituent of lung elastolytic activity and also potently stimulates mucus secretion (4). However, the molecular mechanisms connecting neutrophil elastase and mucus production have been poorly elucidated. In this study, we found that administration of elastase into mouse lungs caused COPD-like phenotypes along with pendrin expression (Fig. 4), just like IL-13. Interestingly, a recent study showed that in addition to IL-4, the inflammatory cytokine IL-1β can induce expression of pendrin in BECs (40). Because elastase induces a strong inflammatory response in the lungs, elastase may induce such inflammatory mediators that in turn cause pendrin expression.

Given that proper control of mucus production is critical to alleviate the symptoms and decrease mortality from bronchial asthma (2, 4), pendrin may be a promising therapeutic target for this disease. Consistent with a recent study (40), we found that niflumic acid, which can inhibit pendrin activity (37), only partially inhibited anion transport and mucus production induced by anion transporters including pendrin (Fig. 6). Pedemonte et al. (40) mentioned that there is no available compound to inhibit the function of pendrin, having screened the Spectrum Library, which contains 2000 compounds. Further understanding of the molecular mechanisms of pendrin's function would pave the way for successful treatment of chronic inflammatory airway diseases such as bronchial asthma and COPD in the future.

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Disclosures

The authors have no financial conflicts of interest.

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