Update in Asthma 2010

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The prevalence of asthma has been increasing, especially in children (1). An article reported that in Canada one in three patients would have a physician diagnosis of asthma in their lifetime (2). Work in the field of asthma this year included evaluation of new therapies, reevaluation of the benefits and risks of available therapy, and research into clinical and biomarker patterns that might allow us to differentiate among different phenotypes of asthma. There were also reports on advances in the understanding of potential immune, cellular, and biochemical mechanisms that might contribute to asthma pathobiology, work in defining genetic contributors to asthma, and elucidation of environmental factors that can influence the development or expression of the disease.

ASTHMA TREATMENT

Long-Acting β-Agonists: Safety and Alternatives

The controversy regarding the safety of long-acting β-agonist (LABA) use in asthma continued. Most data suggest that LABAs alone (without inhaled corticosteroids [ICS]) increase the risk of severe asthma-related events (3, 4). Some metaanalyses suggested little with which to be concerned when LABAs are used with ICS (5, 6), whereas others suggested that the risk persists (7). Regardless, the Food and Drug Administration issued recommendations that LABAs be used only in combination with ICS and for the shortest duration possible (8). The important unanswered questions about LABA use remain: What are the risks of their use in combination with ICS, and how do we balance them against their effects on asthma symptoms? The Food and Drug Administration is working with the manufacturers of LABAs to begin very large studies to answer these questions. Meanwhile, Bleecker and colleagues examined whether the polymorphism at the 16th position of the β_2-adrenergic receptor (ADRB2) modified the response to LABAs (9). Retrospective data had suggested that they might (10), but other studies have not (11). Bleecker and colleagues (9) did not find a pharmacogenetic effect of the polymorphism on differences between LABA monotherapy and combination therapy with ICS. However, the data suggested that subjects who were homozygotes for the arginine (Arg) allele may be at greater risk of developing an asthma exacerbation in the absence of controller medications.

Persistent concerns about LABAs have increased the interest in ascertaining alternatives. In TALC (Tiotropium Bromide Step-Up Therapy for Adults with Uncontrolled Asthma), the Asthma Clinical Research Network of the National Heart, Lung and Blood Institute (NHLBI) studied patients whose asthma was inadequately controlled on ICS who were randomized to doubling the dose of ICS, adding salmeterol, or adding tiotropium (12). Both tiotropium and salmeterol produced greater improvement in peak flow and FEV_1 and better symptom control than doubling the dose of the ICS. Tiotropium was at least as effective, or more effective, than salmeterol for all outcomes examined. These data suggest that anticholinergics, previously dismissed as being inadequately effective in asthma, may represent a viable additional add-on strategy to ICS. Whether tiotropium is associated with severe asthma exacerbations or deaths remains to be determined.

In the same vein, the NHLBI's Childhood Asthma Research and Education network compared the efficacy of a leukotriene receptor antagonist, LABA, and increased ICS in children with asthma suboptimally controlled on low-dose ICS (13). They found that a greater proportion of patients "preferred" (by a hierarchy that included exacerbations, symptoms, and FEV_1) salmeterol as compared with montelukast or ICS. In another study, Vaessen-Verberne and colleagues found that adding a LABA (combination therapy) appeared to be at least as effective as doubling the dose of ICS as judged by time to first exacerbation in children when used for 6 months (14). However, twice as many moderate or severe exacerbations occurred in the LABA-ICS group compared with the doubling-dose ICS group, which again adds doubt about the safety of LABAs in the setting of concomitant ICS therapy in children.

New Therapies

Evaluations of new interventions for asthma, such as bronchial thermoplasty (BT), antibody to the interleukin IL-4 receptor α, parenteral leukotriene modifier, and vitamin D supplementation, appeared this year. BT is a bronchoscopic procedure in which controlled thermal energy is applied to the airway wall with the rationale that decreasing the amount and/or contractility of airway smooth muscle may provide a means to ameliorate the symptoms of asthma (15, 16). In a sham-controlled study in 288 patients published this year, BT led to a clinically small but statistically significant mean positive effect on asthma-related quality of life (0.19 points difference) (17). Seventy-nine percent of thermoplasty subjects versus 64% of sham subjects achieved changes in asthma-related quality of life of 0.5 or greater. There appeared to be a decrease in exacerbations during the observation period after the bronchoscopies, but this was countered by an increase in adverse events and hospitalizations (including pneumonias and an episode of hemoptysis requiring bronchial artery embolization) in the thermoplasty group. None of the secondary outcomes were affected, which included asthma control indices and lung function. Whether a prolonged salutary effect beyond 1 year will be maintained needs to be determined in a randomized study.

This year the results of a study blocking IL-4 and IL-13 signaling in asthma were published in the *Journal*. Corren and

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colleagues were unable to establish the efficacy of an antibody to the IL-4 receptor α (which is responsible for signal transduction for both IL-4 and IL-13) on asthma control over 12 weeks (18). However, a dose-dependent reduction in IgE concentration and a trend toward reduction in asthma exacerbations suggested some possible biological effects.

Although intravenous montelukast administered during an acute asthma attack in an emergency room setting increased FEV_1 at 60 minutes post-dose by (merely) 100 ml compared with placebo, treatment failure (defined as hospitalization or lack of decision to discharge by 3 h post-administration) did not differ between groups (26.8 vs. 29.9%) (19). Thus, parenteral montelukast is not being developed any further for asthma therapy.

Studies have suggested that vitamin D insufficiency may play an important role in the asthma diathesis. While investigating the effect of 1,200 IU/d of vitamin D3 supplementation on reducing the incidence of seasonal influenza A in school children, Urashima and colleagues noted that asthma exacerbations occurred in only two children receiving vitamin D3 compared with 12 children receiving placebo over the 4-month trial period (relative risk, 0.17; $P = 0.006$) (20). In another study, Sutherland and colleagues found an association between serum vitamin D levels and parameters of asthma severity *in vivo* and to treatment response to glucocorticoids *ex vivo* in 54 subjects with persistent asthma (21). The NHLBI's AsthmaNet investigators are preparing to launch a prospective randomized placebo-controlled study of the efficacy of high-dose vitamin D in optimizing asthma control in patients with asthma with symptoms on low-dose ICS therapy (NCT01248065).

PHENOTYPES IN ASTHMA

It is becoming clearer that different pathobiological mechanisms may play a role in different subsets of asthma. Identifying these subsets may help to guide therapy. In an unsupervised hierarchical cross-sectional analysis of 726 subjects, the NHLBI's Severe Asthma Research Program (SARP) investigators identified five clusters (22). Two of these were relatively mild. Two clusters were more severe but differed in their degree of fixed airway obstruction. Interestingly, their analysis revealed a fifth cluster, which consisted of moderate to severe asthma characterized by a preponderance of nonatopic, obese women with late-onset asthma. Whether these clusters differ biologically or in response to therapy will need to be determined. Along similar lines, a cluster analysis by Simpson and colleagues in children from a population-based birth cohort suggested that phenotyping based on timing (early vs. late) and number of atopic sensitizations predicts asthma severity and health care use (23).

Several attempts were made this year to investigate the ability of fractional exhaled nitric oxide (FENO) to identify inflamed airways. Although FENO levels did not distinguish between nonsevere and severe asthma, the Severe Asthma Research Program investigators were able to identify a group of more reactive, at-risk severe asthma phenotype when FENO levels were greater than 35 ppb (24). In another study, Tossa and colleagues found that an increase in FENO levels over 15 months was associated with a twofold increase in bronchial hyperresponsiveness to inhaled methacholine among a population of 351 healthy bakery and hairdressing apprentices (25). Thus, FENO measurements could be useful in the screening of bronchial hyperresponsiveness in workers newly exposed to agents known to cause occupational asthma. Chawes and colleagues studied ENO levels in 253 healthy 1-month-old high-risk neonates and found that increased neonatal FENO level was significantly associated with the development of recurrent wheeze in the first year of life (hazard ratio, 2.63) but not thereafter (26). In a population-based study of 1,506 men and women without asthma, Olin and colleagues found that increased FENO levels were associated with an increased risk of developing wheeze during a 4-year follow-up (27). These results suggest that FENO could be used as a screening tool for subclinical airway inflammation in the general population. The clinical usefulness of such an approach is unclear.

Obesity leads to dynamic hyperinflation and reduced respiratory system compliance. Thus, the increased frequency of asthma symptoms and limitation of activities reported by obese patients with asthma may be related to abnormal airway sensitivity or behavior. Deesomchok and colleagues suggested otherwise (28). They found no difference in perception of dyspnea between obese and nonobese patients with asthma for a given FEV_1 or inspiratory capacity, even after adjustments for body mass index and sex.

Finally, in a large clinical trial, patients with moderate to severe asthma with symptomatic gastroesophageal reflux disease who were placed on 40 mg of esomeprazole once or twice daily demonstrated statistically significant but small improvements in lung function (29). Although the effects for the group as a whole were not particularly impressive, the fact that lung function improved suggests that a subset of patients may actually have functional impairment related to reflux.

ASTHMA PATHOBIOLOGY

Th2 Mechanisms

Th2-type inflammation is a major characteristic of asthma. Attempts to understand how this pathway might be manipulated in animal models was a common area of investigation in asthma this year. Damayanti and collaborators investigated the role of OX40 (a member of the tumor necrosis factor [TNF] receptor superfamily) in sensitization to allergen (30). OX40 and its ligand OX40L play a key role in stimulating helper activity of $CD4^+$ T cells and optimal generation of antigen-reactive effector $CD4^+$ T cells. This report, using anti-OX40L in allergen-exposed mice, suggested that the OX40 costimulatory pathway acts to produce allergen-specific sensitization in primary inhalational exposure to an aeroallergen and also acts on natural killer T cells to produce allergic disease symptoms on secondary exposure. Shutze and colleagues showed that mycotoxins increase the Th2-type response to ovalbumin in the murine allergen inhalation model (31). Their data suggested that this effect was mediated by affecting dendritic cell function that resulted in reduced IL-12 production in dendritic cells and diminished IFN-γ production in T cells. Dougherty and colleagues found that intraepithelial mast cells are increased in subjects with Th2-high asthma, have an unusual protease phenotype, and predict responsiveness to ICS (32). They also found that mast cell gene expression in the airway epithelium related closely to the expression of IL-13 signature genes and thus concluded that IL-13–stimulated production of stem cell factor by epithelial cells potentially explains mast cell accumulation in Th2-high asthmatic epithelium. In another study, Naus and colleagues demonstrated that expression of A Disintegrin And Metalloproteinase (ADAM) 8 plays a role in recruitment of inflammatory cells, including T cells, eosinophils, CD11b1, CD11c2, and CD11c1 cells into the murine airway in this mouse asthma model (33).

In a widely cited article, Saenz and colleagues (34) suggested that IL-25 may also have a role in promoting the accumulation of a lineage-negative multipotent progenitor cell population that represents a previously unrecognized innate immune pathway that promotes Th2 cytokine responses at mucosal sites. Aguilar-Pimentel and collaborators found that allergen-specific $CD8^+$ T cells seem to protect from allergic inflammation in the lungs (35). The number of $CD8^+$ cells seemed to be dependent on

the sensitization dose and appeared to be a critical predictor for the severity of the allergic phenotype.

Cabral and associates reported a novel method to prevent differentiation of T cells into Th2-type cells (36). They demonstrated that interfering with Ca_v 1, a voltage-dependent calcium channel, could prevent inflammation in a murine ovalbumin challenge model. Rao and coworkers showed that blockade of leukotriene B_4 formation might affect the recruitment of $CD4^+$ and $CD8^+$ T cells in the lung after murine allergen challenge (37). Meanwhile, Murdoch and Lloyd reported that γ-delta T cells, a distinct T-cell lineage at mucosal surfaces that are triggered by pattern recognition and endogenous stress signals, are principal producers of IL-17 (rather than Th17 cells) (38). In a murine allergen model they showed that these γ-delta T cells (through IL-17) are critical for effective resolution of airway inflammation.

Toll-like receptors (TLRs) are an important component of the innate immune system that mediate recognition and response to microbial and viral pathogens. Stimulation of the TLR system has been shown to blunt Th2 responses. In a mouse intranasal allergen model, Xirakia and associates showed that triggering of TLR7 appeared to provide long-lived suppression of the Th2 response that appeared to be mediated by type I interferon and also by immunoregulatory CD8 cells (39). Stimulation of this "alternate" immune pathway may provide another method to down-regulate Th2-type responses.

Last, Li and associates contributed to our understanding of possible mechanisms of steroid resistance (40). In a set of *in vitro* experiments, Li and colleagues showed that the isoform of the glucocorticoid receptor (GCR) α, GCR β, may inhibit corticosteroid effects through control of the glucocorticoid response elements of the histone deacetylase 2 promoter.

Non-Th2 Processes

Although Th2-mediated inflammation appears to play a large role in asthma pathobiology, non-Th2 pathways may play an important role as well. Gregory and colleagues showed that overrepression of transforming growth factor-β signaling molecule Smad2 produces increased airway reactivity and airway remodeling after aeroallergen challenge in mice (41). This effect occurred without evidence of "classic" Th2-type inflammation (IL-13 and IL-4) but rather was associated with, and somewhat dependent on, overexpression of IL-25 (IL-17E) mediated through activin A. These data suggest a role for altered epithelial genes in airway hyperreactivity and remodeling. Meanwhile, Lajoie and colleagues demonstrated that severe airway hyperresponsiveness in susceptible mice is driven by dysregulated anaphylatoxin control of the IL-23–Th17 axis leading to excessive IL-17A production (42). They found that the aberrant Th17 response occurs as a result of a shift from complement (C)5a-driven tolerance toward C3a-driven Th17 responses at the airway surface due to either genetic alterations in complement genes or activation of the C3a pathway by environmental triggers of asthma. They suggest that modulation of anaphylatoxins may hold promise for the treatment of asthma.

In addition to cellular inflammation, structural remodeling of the airways is believed to contribute to the airway narrowing and hyperresponsiveness seen in asthma. Ramos-Barbon and colleagues showed that airway smooth muscle proliferation and infiltration by T cells was proportional to asthma severity (43). In another study, Dekkers and colleagues showed that altering the interaction of airway smooth muscle with the extracellular matrix can affect smooth muscle proliferation and airway hyperresponsiveness in response to repeated allergen challenge in the guinea pig (44). This effect was achieved using an integrin-blocking peptide and thus inhibiting the interaction of the smooth muscle with the matrix. Kicic and collaborators (45) reported a series of experiments that suggested that reduced fibronectin production may play a role in the epithelial injury seen in the airways in asthma.

An additional feature of airway remodeling is angiogenesis. Tumstatin is a component of the noncollagen domain-1 of the α3 chain of the collagen IV molecule that inhibits angiogenesis via blockade of the interaction of vascular endothelial growth factor with the $\alpha_v\beta_3$ integrin, causing apoptosis of proliferating endothelial cells. In a study of bronchoalveolar lavage in humans, Burgess and associates showed that the level of tumstatin is decreased 18-fold in the airways of patients with asthma but not in subjects without asthma (46). Additionally, in a chronic ovalbumin-induced allergic airways disease mouse model, tumstatin inhibited angiogenesis and accumulation of vascular endothelial growth factor in the airway and decreased airway hyperresponsiveness.

This year we also gained increased insight into possible mechanisms through which chitin and chitinases might play in promoting inflammation operative in asthma. Chitin is a polysaccharide present in fungi, insects, allergens, and parasites. Chitinases have been associated with asthma and asthma severity. Da Silva and colleagues demonstrated that chitin particles can act as adjuvants for ovalbumin sensitization in mice that skew the inflammatory response to Th2-type inflammation (47). In exploring the ability of the antiinflammatory Clara cell 10-kD (CC10) protein to modulate eosinophilic chronic rhinosinusitis, Wang and associates showed that CC10's ability to down-regulate eosinophilic chronic rhinosinusitis inflammation occurs by reducing the expression of Chitinase 3-Like 1 (CHI3L1) (48). Furthermore, they showed that anti-CHI3L1 reduced eosinophilic inflammation in this model. Additionally, work by Wu and coworkers further reinforces a role for chitinases in clinical asthma (49). They showed a relationship between exacerbations and polymorphisms in the chitinase gene CHIT1. These data reinforce our suspicions that the chitin-chitinase axis may be an important genetic and environmental contributor to asthma.

Last, genome-wide expression profiling surprisingly suggested that an apolipoprotein E–low-density lipoprotein receptor pathway may play a role in murine house dust mite–induced asthma models. Based on this information, Yao and colleagues went on to demonstrate that this pathway may act as an endogenous negative regulator of airway hyperresponsiveness and goblet cell hyperplasia in asthmatic airways (50). This unexpected finding suggests a role for the low-density lipoprotein receptor pathway as a therapeutic target in asthma.

Microorganisms in Asthma

It has been hypothesized that infectious agents might play a role in initiation or perpetuation of asthma. This year studies have examined the role of fungi, atypical organisms, and the airway's microbiome itself. Fairs and colleagues found an association between *Aspergillus fumigatus* colonization or sensitization and lung function in 72 patients with asthma who were ruled out for allergic bronchopulmonary aspergillosis (51). Using molecular analysis of the polymorphic bacterial 16S-rRNA gene, Hilty and colleagues characterized the microbiota of asthmatic airways and compared them with healthy control airways (52). They found that the bronchial tree was not sterile in either group of subjects. It contained a mean of 2,000 bacterial genomes/cm^2 surface sampled. They also found that asthmatic airways were characterized by pathogenic Proteobacteria, particularly *Haemophilus* species. Although robust, these associations do not yet establish causality between the presence of

pathogens and asthma. Meanwhile, Sutherland and colleagues were unable to find evidence for the atypical bacteria *Mycoplasma* and *Chlamydophila pneumoniae* by performing polymerase chain reaction techniques on endobronchial biopsies from 92 patients with suboptimally controlled asthma on ICS therapy (53). Contrary to their hypothesis, macrolide therapy did not provide any antiinflammatory benefits in these subjects. To study the progression of an upper respiratory illness into lower respiratory tract inflammation and examine the efficacy of macrolides in preventing such progression, the NHLBI's AsthmaNet is preparing to launch a study examining this in preschool children with history of recurrent severe wheeze (NCT01272635).

ASTHMA AND GENETICS

The degree of heritability in asthma suggests a significant genetic component in its etiology. Genome-wide association studies (GWAS) represent a hypothesis-free powerful approach to identify genes that might contribute to this heritability. Moffatt and colleagues conducted a GWAS by genotyping 10,365 persons with physician-diagnosed asthma and 16,110 control subjects recruited from 23 studies, all of whom were matched for ancestry (54). This study implicated an association between asthma, IL1RL1/IL18R1, HLA-DQ, IL33, SMAD3, and IL2RB. These genes are involved in communication of epithelial damage to the adaptive immune system. They found that the association with the ORMDL3/GSDMB locus on chromosome 17q21 previously identified by others was specific to childhood-onset disease. Sleiman and colleagues identified a locus of asthma susceptibility that conferred asthma susceptibility both in individuals of European and African ancestry (55). This locus contains DENND1B, a gene expressed by natural killer cells and dendritic cells that is predicted to interact with the TNF-α receptor. Both of these studies reconfirmed the association between asthma and the ORMDL3/GSDMB locus on chromosome 17q21.

Although both the GWAS studies mentioned above found susceptibility genes that seemed to cross races, race can certainly confound these types of genetic studies and can affect other asthma outcomes. This year Kumar and colleagues reported that using ancestry informative markers to determine percentage African American ancestry was better than self-identified race in more accurately predicting FEV_1 (56). They found that self-identified race alone might result in a misestimation of asthma severity (in both directions) among 1 to 4% of those who identify themselves as African American. An article by Fritz and colleagues suggests that additional factors can complicate assessments by race or ethnic group (57). They reported that Latino children with asthma are less accurate at subjectively assessing their lung function status than non-Latino white children. They tended to overestimate their degree of compromise.

Sharma and colleagues examined differentially expressed genes between different stages of human fetal lung development (58). Among more than a 1,000 target genes were five genes of the Wnt pathway that has been shown to play a role in lung development in murine models. They subsequently showed that single nucleotide polymorphisms in several of these genes were associated with lung function in several children's asthma cohorts. Such methods may allow us to home in on additional promising candidate genes for asthma.

ASTHMA AND THE ENVIRONMENT

It is clear that the development of asthma involves an interaction between genetic predisposition and environmental stimuli. Wang and colleagues showed that subjects exposed to high–molecular weight (MW), low-MW, and mixed environmental agents were at a higher risk of asthma than those who were not (59). Furthermore, they showed that high MW exposure was associated with induction of atopic asthma, whereas low MW was more associated with nonatopic asthma. Another article in the *American Journal of Respiratory and Critical Care Medicine* found positive associations between traffic-related air pollution levels outside subjects' homes and the incidence and prevalence of asthma during the first 8 years of life (60). In another publication, the authors showed that even at relatively low ambient concentrations, ozone and primary pollutants from traffic sources independently contributed to the burden of emergency department visits for pediatric asthma (61). Although not as preventable, another article in the *American Journal of Respiratory and Critical Care Medicine* reported that exposure to significant amounts of desert dust was found to be associated with increased risk of asthma admission in children (62).

An example of the potential remarkable effect of an environmental intervention was highlighted by Mackay and colleagues (63) in their report of the effect of public smoking cessation legislation in Scotland. They found that after such legislation was enacted, the rate of pediatric hospitalizations decreased by greater than 18% per year. Wright and colleagues demonstrated that "environment" can extended to include prenatal maternal stress, which may alter both adaptive and innate immune axes (64).

SUMMARY

This year was the year of nature more than nurture for asthma. We saw a greater understanding of the effect of environmental influences, even at low levels, on asthma morbidity. More profoundly, we came to understand that interventions such as reducing smoking could have dramatic effects on health care use related to asthma. Although new therapies were disappointing, new therapeutic approaches and biologics are in phase II testing, and the effectiveness of these targeted interventions will become available over the next few years. The fruits of genetic investigations are yet to be realized, but they are beginning to influence our investigations into the pathobiology of asthma. There remains much to be done in differentiating the various pathobiological processes that may cause individuals to wheeze.

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