Pulmonary, Sleep, and Critical Care Updates

Update in Asthma 2009

Wendy C. Moore¹ and Rodolfo M. Pascual¹

¹Center for Genomics and Personalized Medicine and Section on Pulmonary, Critical Care, Allergy and Immunologic Diseases, Wake Forest University School of Medicine, Winston Salem, North Carolina

The emergence of a novel H1N1 influenza virus in early 2009 dominated the headlines and caused much fear worldwide as the Centers for Disease Control (CDC) proclaimed a pandemic (1). The media's preoccupation with the H1N1 epidemic, however, meant less "on-air" time for debates on the safety of asthma medications, a focus of last year's update (2). Ironically, clinical asthma trials this year also shifted away from the usual asthma medications toward the use of alternative "nonasthma" medications to treat comorbidities (3, 4) or as immunomodulators (5) with the goal of improved asthma control (decreased impairment). The importance of asthma exacerbations as a marker of poor asthma control and as a risk factor for poor long-term outcomes was investigated (6). Other studies examined the prediction of and effective treatment of exacerbations (7, 8). Challenges in the treatment of severe asthma were explored (9) and novel monoclonal antibodies were studied (10–12) as potential treatment options for patients with the most severe disease. Articles published in the *American Journal of Respiratory and Critical Care Medicine* in 2009 advanced our understanding of the influence of genetics (13–20), gene regulation (19, 21–23), factors in early life (24–28), and the environment (24, 29–33) on the development of asthma or the modification of disease severity. Basic pathobiological studies in humans (22, 23, 30, 34–44) and in animals (21, 29, 36, 45–56) added to our understanding of specific mechanisms in different phenotypes of asthma. Overall, a few areas emerge as particular highlights of the year and we focus your attention to the topics below.

THE H1N1 EPIDEMIC AND ASTHMA

Early on it became apparent that this novel strain of influenza exhibited unique epidemiological features causing severe disease and death in children and young adults in contrast to the usual seasonal strains that cause the most morbidity in the elderly (1, 57–60). Based on the limited clinical data available early in the epidemic, the CDC identified patients with asthma as an at-risk group for serious illness from this infection. There are several pathophysiological mechanisms that could lead to increased susceptibility of patients with asthma to the H1N1 virus, including impaired epithelial function, altered responses to vaccine due to chronic corticosteroid therapy (61, 62), or

perhaps impaired T cell-mediated immunity. There is some evidence that persistent or more vigorous viral replication occurs in those who are more seriously affected and in those with asthma (62). In the clinical arena, asthma is a frequently reported comorbidity in patients hospitalized with H1N1 infection, especially in critically ill patients (60, 63). From the start, the CDC designated asthma as a priority group for vaccination, but compliance with this recommendation is unknown.

Historically, vaccination rates for seasonal influenza in the asthma population have been abysmal (64), with doctors and patients citing a variety of reasons for nonadherence to guidelines. There has been controversy over efficacy of the vaccine in certain age groups (64–66). There remain persistent, unsubstantiated concerns about the risk of exacerbation of asthma in adults after influenza vaccine administration despite evidence to the contrary (66, 67). There is uncertainty about the serologic response to standard doses of vaccines in patients with asthma, particularly those taking corticosteroids (61). Several combined efforts at studying vaccines in asthma are underway, including one study sponsored by the National Heart Lung and Blood Institute (NHLBI) and National Institute of Allergy and Infectious Disease, wherein investigators from the Severe Asthma Research Program have conducted a vaccine study in patients with severe asthma. These studies will provide guidance in the development of vaccination strategies for patients that will maximize immunogenicity while minimizing adverse effects. Whether or not the current vaccination campaign will improve adherence rates or effectively protect the population from future widespread H1N1 outbreaks remains unknown at this time.

ALTERNATIVE (NONASTHMA) DRUGS FOR ASTHMA

Gastroesophageal Reflux Disease and Asthma

Two previous randomized, placebo-controlled clinical trials have shown that treatment with a proton pump inhibitor (PPI) reduced nocturnal symptoms (68), decreased asthma exacerbations, and improved quality of life measures (69) in patients with symptomatic gastroesophageal reflux disease (GERD). Based on these data, the 2007 National Asthma Education and Prevention Program Guidelines for the Diagnosis and Management of Asthma recommend medical management for GERD in patients with symptoms of reflux and suboptimally controlled asthma (70). In the Study of Acid Reflux and Asthma (SARA), the American Lung Association–Asthma Clinical Research Centers studied the prevalence of asymptomatic GERD ("silent reflux") in moderate to severe asthma and the effect of treatment with a PPI on asthma control (3, 4). SARA was a large (n = 412) randomized double-blind 24-week clinical trial of esomeprazole compared with placebo in patients with inadequately controlled asthma (Asthma Control Questionnaire score > 1.5 or an exacerbation in the past year) on medium to high doses of inhaled corticosteroids (ICS). The primary outcome was episodes of loss of asthma control

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Correspondence and requests for reprints should be addressed to Wendy C. Moore, M.D., Wake Forest University School of Medicine, Center for Human Genomics, Medical Center Boulevard, Winston Salem, NC 27104. E-mail: wmoore@wfubmc.edu

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(30% decrease in peak expiratory flow rate, urgent care visit, or need for oral corticosteroids); 42% of patients met this definition during the trial. There was no improvement in this outcome, nor any secondary outcome (lung function, asthma symptoms, or quality-of-life measures) with PPI therapy. Nearly half of the patients did indeed have silent reflux documented by ambulatory pH monitoring, but PPI therapy showed no benefit in this subgroup of patients either. An additional finding was that there was no difference in baseline clinical characteristics between the patients with silent reflux by pH probe and those without acid reflux events, suggesting that asymptomatic GERD was not associated with greater disease severity or worse asthma control before randomization (3). The SARA study concludes that asymptomatic GERD, although present in many patients with uncontrolled moderate to severe asthma, is not associated with poorer asthma control, and thus treatment of this entity does not improve control. Given the cost of PPIs, it seems reasonable to consider evaluation and treatment in patients with symptoms of GERD, but the SARA study does not support this approach in those without symptoms.

Antibiotics as Immunomodulators in Asthma

There has been considerable interest in the use of macrolides in asthma for more than 15 years. A recent review of long-term macrolide therapy in chronic obstructive airway diseases reiterates the findings of a prior metaanalysis: there is insufficient evidence to conclude benefit from macrolide therapy in chronic asthma (71, 72). If macrolide therapy is efficacious, however, a major research question is whether only those with evidence for latent *Chlamydia pneumoniae* or *Mycoplasma pneumoniae* infection would improve (an antibacterial mechanism) or whether it is the immunomodulatory properties of the drugs that are responsible for a clinical effect, so that even those not infected might respond. The Asthma Clinical Research Network has recently completed the Macrolides In Asthma trial, a randomized, double-blind clinical trial of clarithromycin compared with placebo in patients stratified by the presence or absence of polymerase chain reaction positivity for these organisms in bronchial mucosal samples. This trial should shed some light on the prevalence of these organisms in the airways of patients with asthma, the clinical effects of therapy in those both with and without polymerase chain reaction–positive specimens, and the biologic mechanisms that might underpin these responses.

This year an antifungal agent was studied in a scenario much like the macrolide studies, as a potential treatment for sub-clinical infection or perhaps as an immunomodulator in asthma. The Fungal Asthma Sensitization Trial (FAST) was a small ($n = 58$) 48-week single-center, randomized, double-blind clinical trial investigating the effect of treatment with itraconazole on quality-of-life measures in patients with severe asthma (5). Asthma in these patients was indeed quite severe, with frequent exacerbations and oral corticosteroid bursts despite chronic high-dose ICS or oral corticosteroid therapy. All patients showed skin-prick positivity to fungal allergens. Patients who received itraconazole showed an average improvement of 0.85 (95% confidence interval; 0.28, 1.41) in their Asthma Quality of Life Questionnaire (AQLQ) score as compared with a -0.01 ($-0.43, 0.42$) change in the placebo group ($P = 0.02$). The patients who completed 32 weeks of antifungal therapy continued to improve; 61% of the group further increased their AQLQ score more than 0.75 ($P = 0.01$). This improvement in AQLQ is similar to that seen with omalizumab therapy (73). Many of the patients in the FAST study likely would have been candidates for omalizumab therapy given their medication

requirements, health care use, and serum IgE levels. Although the antifungal properties of itraconazole might explain the results of the FAST study, it is also possible that the drug is functioning as an immunomodulator in a paradigm similar to that seen with macrolide antibiotics. A repeat study of similar patients without fungal sensitization would be an interesting follow-up study that might unmask an immunomodulator effect independent of the antifungal activity.

MONOCLONAL ANTIBODIES FOR SEVERE ASTHMA

Anti-Tumor Necrosis Factor in Severe Asthma

In 2006 several articles suggested promise for antagonism of the tumor necrosis factor (TNF) axis in patients with severe corticosteroid-refractory asthma (74, 75). This year, a Phase II multicenter randomized dose ranging study of golimumab, an anti-TNF monoclonal antibody, was terminated early (at 24 wks) secondary to apparent lack of efficacy and safety concerns due to an observed increase in serious infections, especially pneumonia, in the treatment group (10). The golimumab study enrolled 309 subjects with severe persistent asthma defined by treatment with high doses of ICS and a long-acting β -agonist (LABA) and two or more asthma exacerbations in the past year. Asthma in these patients was very severe; one-third were on chronic oral corticosteroids and the average baseline pre-bronchodilator FEV₁ was 60% predicted. The study failed to show benefit of the drug over placebo with regard to lung function (FEV₁) or exacerbation rates. In *post hoc* analyses to identify responder subtypes, however, there was a favorable treatment effect (decrease in severe asthma exacerbations) in three subgroups: subjects with onset of asthma after the age of 12 years (odds ratio [OR], 0.40; $P = 0.03$), those with a reported history of sinusitis (OR, 0.40; $P = 0.02$), and those with a 12% or greater change in FEV₁ after four puffs of albuterol (OR, 0.30; $P = 0.01$). Taken together these three subgroups have common elements that would suggest a previously described severe asthma phenotype: patients with late-onset asthma, frequent sinopulmonary infections, and low prebronchodilator lung function (and thus greater ability mathematically to reverse more than 12%) (76–78). These patients tend to be less atopic and this phenotype has been associated with neutrophilic airway inflammation, theoretically making these patients less likely to respond to therapies directed toward IgE- or eosinophil-mediated mechanisms. A follow-up study investigating the effect of anti-TNF drugs on these possible responder subgroups should be considered because therapeutic options are severely limited in this difficult group of patients. A *post hoc* analysis of the golimumab data could identify a subgroup of subjects at risk from the drug who should avoid these agents. Future studies of anti-TNF agents will have to balance the potential risks against the potential benefits of these drugs for patients with the most severe asthma.

Anti-IL-5 in Severe Asthma

This year two randomized, double-blind trials studied the effect of mepolizumab, an anti-IL5 monoclonal antibody, in the treatment of severe corticosteroid-refractory asthma (11, 12). Both studies proved the basic pathobiologic concept that an anti-IL5 antibody can rapidly decrease blood and sputum eosinophils after just one or two doses of the drug in individuals with elevated sputum eosinophilia. The larger trial ($n = 61$) studied the effect of mepolizumab as add-on therapy in patients with severe asthma on high doses of ICS/LABA or chronic oral corticosteroids (53–57% of patients in this study) with frequent exacerbations (mean = 5/yr) and persistent sputum eosinophilia

(>3%) (11). At the end of the 52-week treatment period, the mepolizumab group had fewer asthma exacerbations (OR, 0.57; $P = 0.02$), the primary outcome variable. There was no significant effect on secondary outcomes, such as patient-reported symptoms and lung function measures, although the AQLQ marginally improved by 0.55 (minimal clinically important difference 0.5) in the mepolizumab group ($P = 0.02$). This pattern of response in outcome variables (decreased exacerbation rates without changes in lung function) was also seen in the omalizumab trials (73, 79, 80) and suggests that immunomodulators are more likely to decrease risk (exacerbations) than to change traditional markers of control (lung function, symptoms). The results of the mepolizumab study emphasize another important point about outcomes in severe asthma trials: these medications are unlikely to eliminate exacerbations (risk). Although greatly reduced, there were still an average of two severe exacerbations per patient-year in the mepolizumab-treated group. This is a remarkable improvement in exacerbation rate for a group of patients with limited therapeutic options and should not be undervalued. Patients with severe asthma will always have exacerbations; we just need to minimize them.

PATHOBIOLOGIC MECHANISMS IN ASTHMA

Beyond Th2 Cells

Signaling and inflammatory cascades important in asthma continue to become more complex as new information from human and animal models has led to the identification of novel regulators of the Th2 lymphocyte inflammatory pathways. This year several studies identified novel regulators of Th2 cells in asthma (36, 46, 49, 51, 81). One evolving paradigm is that effector cells responsible for asthma inflammation are regulated not just by Th2 lymphocytes, but by a cadre of T-helper cells including those of the Th1 (82–85), IL-17–producing T cell (Th17) (50, 55, 86–88), and regulatory T cell (Treg) (37, 41, 48, 52, 89, 90) cell types. It has been known for several years that Th1 cells tend to antagonize the functions of Th2 cells and recent data suggest that a deficiency of Th1 types of signals can lead to up-regulation of Th2-mediated inflammation. Elegant work performed in a human model demonstrated distinct molecular phenotypes in asthmatic airways while showing that it is possible to profile the pattern of T-helper cell–mediated inflammation *in vivo* (43). Similarly, Tregs, which have been implicated in autoimmunity and the regulation of immune tolerance, have been recognized as generally inhibiting Th2 responses in asthma. Interestingly, Treg cells are responsive to environmental exposures via innate immunity mechanisms, which have also been linked to asthma (18). Several studies cited herein demonstrate that Th17 cells that are important in adaptive immune responses also interact with and antagonize Th2 cells to regulate airway inflammation. The balance of cytokines produced by the various types of T cells may alter susceptibility to infection and hence to asthma in early life (28). Taken together, the results of these studies drive home the point that an imbalance of T-cell subtypes may be responsible for the perpetuation of asthma at least in some patients.

Asthma Genetics and Genomics

There were several studies of the genetics and genomics of asthma in the *American Journal of Respiratory and Critical Care Medicine* in 2009. Notably, one study demonstrated associated polymorphisms in the promoter region of the gene encoding chitinase 3-like 1 (*CHI3L1*) associated with atopy and increased serum levels of YKL-40 (19), a previously reported candidate

biomarker of asthma severity and airway remodeling (91, 92). Several gene–asthma associations reported previously were replicated in novel study populations using genome-wide association scanning and other approaches (13, 18, 93–96). The association of asthma phenotypes with genetic variability on chromosome 17q12–q21 was replicated in several studies (13, 95, 96). Smit and colleagues replicated the association of the gene encoding CD14 (an innate immunity protein) with asthma in a rural adult population and further determined that there were genes (CD14 and toll-like receptors) by environment (country living) interactions in their cohort (18). Two studies replicated the previously reported single-nucleotide polymorphism–asthma associations in the gene *DPP10* (93, 94) (which encodes dipeptidyl-peptidase 10, a protein that interacts with voltage-gated potassium channels) and in one of these studies a replication of the previously reported asthma–*ADAM33* gene association was also seen (93). In a very large genome-wide association scanning study asthma was associated with eosinophilia and several genetic loci (*IL1RL1*, *WDR36*, *IL33*, and *MYB*) (97). These findings are interesting because a mouse homolog of the *IL1RL1* gene encodes a receptor that may modulate T-cell functions, whereas *IL33* encodes interleukin 33, shown recently to be expressed in higher levels in the airways of patients with severe asthma (98). The relevance of the other genes is less clear as *WDR36* encodes a member of a family of proteins involved in many cellular processes, whereas *MYB* encodes an essential hematopoietic transcription factor that is also an oncogene. Other studies showed genetic associations in candidate asthma pathways (84, 85, 97), associations related to asthma severity or related phenotypes (14, 15), gene–environment interactions (17, 20), or gene–gene interactions (epistasis) (84).

Several papers examined genes associated with pathways targeted by common asthma therapies (99–102). The gene *STIP1*, which encodes a component of the glucocorticoid signaling machinery, was associated with lung function responses to ICS suggesting a possible underlying genetic mechanism as a determinant of steroid responsiveness (100). In the Childhood Asthma Management Program cohort the *PDE4* gene (phosphodiesterase 4, a target of theophylline) was associated with asthma disease susceptibility (101). The Asthma Clinical Research Network published the results of the Long-Acting β_2 -agonist Response by GEnotype (LARGE) study, a genotype-stratified clinical trial ($n = 87$) evaluating the effect of genetic variability in the *ADRB2* gene on response to LABA with concomitant ICS treatment (102). The study showed that treatment with a LABA/ICS combination was superior to ICS alone in improving morning peak expiratory flow rate (primary outcome) regardless of genotype (Gly/Gly or Arg/Arg at the 16th amino acid [codon] of *ADRB2*). These results suggest no pharmacogenetic effect (negative or positive) of this single-nucleotide polymorphism on lung function responses to a LABA in the setting of concomitant ICS use. In a *post hoc* exploratory analysis of African-American subjects ($n = 17$), however, the investigators found that patients with the Arg/Arg genotype did not benefit from the addition of a LABA to ICS treatment. The authors emphasized the exploratory nature of these findings, but they are provocative in light of evidence that response to and side effects of LABA vary by race (103).

Novel Asthma Pathways

The role of the vitamin D pathway in asthma has continued to receive considerable attention. Vitamin D deficiency is very common and previous studies suggest that vitamin D may have a role in the pathogenesis of asthma (104). Vitamin D has been shown to modulate the functions of Treg (90), and polymorphisms in the vitamin D receptor (105) (*VDR*) gene (91) and

other pathway genes (106) have been associated with asthma disease susceptibility. Most recently, Brehm and colleagues studied variability in the 25-hydroxyvitamin D gene in patients with asthma in a family-based genetic study including 616 children aged 6 to 14 years in Costa Rica (107). Although only 28% of these children had vitamin D deficiency (< 30 ng/ml), low vitamin D levels were associated with increased markers of atopy (total IgE, serum eosinophils). In addition, serum vitamin D levels were inversely associated with the risk for hospitalization in the past year for asthma as well as with bronchial hyperresponsiveness. Taken together, these studies suggest that vitamin D deficiency may be associated with asthma susceptibility or disease severity and that genetic variability in the vitamin D pathway genes could modulate this effect.

Numerous prior studies have shown that S-nitrosothiols, such as S-nitrosoglutathione, are relevant signaling molecules in asthma and that similar to the effects of nitric oxide on vascular smooth muscle, S-nitrosoglutathione is a potent endogenous bronchodilator (108). S-nitrosoglutathione is produced by nitric oxide synthase in the presence of glutathione and is degraded by S-nitrosoglutathione reductase. Recently, it was shown that S-nitrosothiol content was decreased, whereas S-nitrosoglutathione reductase activity was significantly increased in the airways of subjects with asthma (38). This net negative "glutathione balance" is of particular interest given the recent association of acetaminophen (paracetamol) intake with asthma (109). Acetaminophen is a commonly used medication known to deplete glutathione stores in cells.

CONCLUSIONS

This past year saw some unusual developments in the arena of clinical asthma. A novel pathogen emerged (H1N1), and as with other respiratory viruses, patients with asthma seemed to be more susceptible to this infection and more at risk for serious illness. The need to implement an urgent vaccination campaign revived old controversies surrounding seasonal influenza vaccination in patients with asthma. We eagerly await the results of the Severe Asthma Research Program's H1N1 vaccine trial and other vaccine studies as they should shed light on the efficacy and safety of influenza vaccines as assessed in a controlled clinical trial setting. As the current wave of H1N1 wanes in the United States, vaccination rates will likely continue to decrease. The results from the National Institutes of Health trial will provide evidence-based recommendations for the future vaccination campaign that will be needed.

The high profile clinical trials in the past year were not the typical asthma studies aimed at fine-tuning the current treatment guidelines. Instead, trials examined the potential for nonasthma medications (not even in the current guidelines) as add-on therapy in moderate to severe asthma and the potential role for new biologics in the development pipeline for those with the most severe disease. Taken together these studies emphasize that there is a heterogeneous group of patients with severe asthma, not all of whom can be controlled with the current medication arsenal that is endorsed by the guidelines. Immunomodulators will be the future of severe asthma treatment whether they are nonspecific oral agents (such as macrolides or azoles, or perhaps vitamin D) or targeted monoclonal antibodies (anti-IgE, anti-TNF, or anti-IL5).

Correct clinical trial design is paramount to finding new medications for severe asthma; one must study the right drug in the correct subset of patients and measure relevant outcomes with realistic expectations. The two monoclonal antibody studies reviewed in this update are prime examples highlighting these issues. The characteristics of subjects identified *post hoc* as

possible responders in the anti-TNF study would not have been intuitive at the time of study design. As a result, selecting the correct patients *a priori* was not feasible and the overall trial was negative. There may well be a role for anti-TNF drugs in severe asthma, especially in later-onset severe asthma with low lung function and frequent sinopulmonary infections, the older less atopic (perhaps neutrophilic) phenotype. Follow-up clinical trials using the lessons learned in the golimumab study should be conducted. Although several anti-TNF agents are currently Food and Drug Administration–approved for use in rheumatologic diseases, the potential risks of these drugs in severe asthma is significant and their use in the clinical arena will need to wait for the results from future clinical trials.

In contrast, the mepolizumab studies benefited from a measurable biomarker that identified a likely responder phenotype *a priori*; the classic atopic, eosinophilic exacerbating asthma phenotype. By selecting the right patients and using a drug that could be proven to affect that biomarker (proof of concept) they were able to show improvement in their primary outcomes (treatment failure and rate of asthma exacerbations). As such, anti-IL5 biologics are well on their way to large phase III clinical trials already. Unfortunately, in the real world things are not always so neat. As clinical experience with the only Food and Drug Administration–approved biologic has grown (omalizumab, anti-IgE), it has become apparent that some patients with an elevated baseline IgE (the predetermined responder phenotype) do not respond to the drug (110). In retrospect it was naive to believe that patients within a responder phenotype identified with limited biomarkers (skin test positivity, IgE) would all be alike. There is heterogeneity within the responder group and thus a continuum of response.

In the fall of 2009, the new NHLBI-sponsored multicenter asthma clinical trials network AsthmaNet began designing novel protocols to explore controversies in pediatric and adult asthma, including severe disease. The importance of asthma heterogeneity has been clear since the inception of the National Institutes of Health asthma networks, and we can expect this group of investigators to identify novel composite asthma phenotypes that will impact clinical asthma care in the future. Understanding asthma heterogeneity is crucial to allow development of responder profiles or to identify patients at risk for individual medications in the future. After all, the challenge is to find the right medication for your severe asthma patient in the future.

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