

Update in Asthma 2006

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Asthma-related investigations reported in 2006 ranged from the characterization of clinical asthma and airway obstruction (1–4), to human biologic studies (5, 6) and the use of animal models to better understand pathobiologic mechanisms at a cellular and molecular level (7–19). The concept of “risk” has been featured prominently, with articles identifying factors that increase the risk of developing asthma (20–24) as well as those aimed at identifying risk factors for asthma exacerbations and adverse outcomes (25–29). Studies have continued to explore the utility of noninvasive biomarkers (exhaled nitric oxide, exhaled breath condensate) to identify (30, 31), modify (32), and treat disease while increasing understanding of pathophysiologic mechanisms in asthma (33, 34). Clinical trials have sought to optimize treatment with established asthma drugs (35–37) and to evaluate novel modalities to fill voids in our current armory of asthma medications (38–40). Overall, this year has been an interesting journey, particularly in clinical asthma research, challenging accepted concepts in asthma therapy, and urging clinicians to expand their assessment of patients to identify and modify patient risk in addition to traditional measures of asthma control (41).

NEW CONCEPTS IN THE TREATMENT OF ASTHMA

Long-Acting β -Agonists, Risk, and the Genetics of the β_2 -Adrenergic Receptor

Long-acting β -agonists (LABAs) came under increased scrutiny this year with the publication of the SMART (Salmeterol Multicenter Asthma Research Trial) study in January 2006 (42–44). The SMART study was a randomized, double-blind, placebo-controlled surveillance study conducted to assess the effect of the addition of salmeterol to “usual asthma pharmacotherapy” on asthma-related outcomes in a large sample of 26,355 subjects with asthma (42). The study was terminated early due to the inability of trial investigators to enroll the projected number of subjects with asthma in a timely manner, and due to the statistically significant increases in the secondary outcomes of respiratory- and asthma-related deaths and life-threatening experiences in subjects treated with salmeterol as compared with placebo. Although this increased risk was present in the entire cohort, *post hoc* analyses of these results showed that this increased risk appeared to be exaggerated in African Americans, especially in those not receiving inhaled corticosteroids (ICS) concurrently. It is important to note that only 25% of subjects in the SMART study were African American and baseline lung function was lower, measures of urgent health care utilization were greater, and fewer subjects were using ICS in this group, suggesting

poorly controlled and possibly inadequately treated asthma at baseline that could have contributed independently to increased risk. Despite these issues, the SMART study contributed significantly to the ongoing U.S. Food and Drug Administration (FDA) evaluation of the safety of LABAs in general and led to the modification of the black box warning on the packaging label for salmeterol and the addition of a black box warning for formoterol, cautioning the use of LABAs either as a single agent or in a combination product as first-line therapy for asthma (45). This FDA recommendation was in stark contrast to previous literature suggesting a preference for a lower dose ICS/LABA combination product over medium doses of ICS alone (46). Despite the FDA warning, it is important to recognize that the recently published 2006 Global Initiative for Asthma (GINA) guidelines (47) continue to recommend a low-dose ICS/LABA combination product (rather than increasing the ICS dose) as the preferred “step up” choice for persistent asthma not well controlled on low doses of ICS alone, an approach supported by numerous randomized clinical trials (46).

The LABA controversy stimulated investigations in reference to genetic variation of the β -adrenergic receptor (ADR β 2) gene (48–53). The single nucleotide polymorphism (SNP) in the ADR β 2 gene that has been best studied clinically results in an arginine (Arg) or a glycine (Gly) at the 16th amino acid. Subjects with an Arg16Arg genotype (the “risk” genotype) have been shown to have lesser (or adverse) clinical responses to short-acting β -agonists (SABAs) (48–50), but the response to LABAs in these subjects has been variable (50). The Asthma Clinical Research Network performed a retrospective analysis of two previous trials to evaluate the effect of the Arg16Arg genotype on the clinical response to the addition of an LABA in subjects already treated with an ICS (51). In both trials, subjects with the Arg16Arg genotype showed adverse outcomes when treated with the LABA salmeterol, and this effect was not abrogated by concurrent treatment with ICS. In contrast, a similar industry-sponsored retrospective analysis of the effect of the Arg16Arg genotype on clinical response to the addition of an ICS/LABA combination product in subjects not well controlled with as-needed SABAs failed to show any difference in baseline pulmonary function or response to fluticasone/salmeterol based on ADR β 2 genotype (52). It is not clear whether the disparity in results between these two analyses is due to larger sample size ($n = 183$) and/or the concurrent start of ICS with the LABA in the latter study. It is important to note, however, that all of the clinical trials analyzed in these reports were primarily aimed at assessing measures of asthma control and clinical response to LABAs, not exacerbations or risk of adverse events, the outcomes assessed in the SMART study (42).

In-depth sequencing of ADR β 2 this year revealed that the genetic variation at the 16th amino acid is only the tip of the genetic iceberg. Hawkins and colleagues reported multiple new SNPs in the ADR β 2 gene in a large admixed cohort ($n = 1,185$, 44% African American) with significant differences in allele frequency among races (53). Although the Arg16Arg genotype was more frequent in African Americans, and some Arg16 haplotypes were present only in African Americans, other genetic

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variations showed positive associations with measures of baseline lung function and bronchodilator reversibility in this racial group. Little is known about clinical responses to β -agonists in African Americans, because there were few subjects of this racial group in the clinical trials analyzed for the effect of the Arg16Arg genotype (51, 52). Could African Americans be at greater risk for inadequate or adverse effects of β -adrenergic agonists (SABAs or LABAs) due to their genetic background, some of which appears to be unique to this ethnic group? Was the increased risk of death and severe exacerbations seen in the African Americans in SMART possibly due to a genetic phenomenon? Ongoing clinical trials will hopefully answer these questions in the future.

New Concepts in the Use of ICS

Regularly scheduled maintenance dosing of ICS continues to be the first-line approach for using controller medications in persistent asthma (46, 47). Clinical trials in 2006 explored two major questions in the use of ICS: (1) Can prophylactic use of ICS in children at risk for developing asthma abrogate onset of disease and (2) can an ICS/LABA combination product be used in an "as needed" manner for rescue in patients already treated with an ICS/LABA (unscheduled "extra puffs" for asthma symptoms)?

ICS preasthma. The role of ICS as "prophylaxis" to prevent the development of asthma in children at risk for the disease was explored in three large clinical trials this year (54–56). The trials differed in their definitions of the "at risk" child, the timing of steroids (in relation to the child's age), and the duration of ICS therapy and follow-up. In the PAC (Prevention of Asthma in Children) study, infants of mothers with asthma were randomized to receive short 2-week courses of budesonide whenever they had a transient wheezy episode of more than 3 days in duration (54). At 3 years of age, there was no difference in the development of persistent wheezing in the children treated with intermittent ICS in comparison with control subjects. The IFWIN (Inhaled Fluticasone in Wheezy INfants) study randomized very young children (mostly < 2 yr old) with an atopic parent and a history of wheezy episodes to treatment with fluticasone (in a step-down manner) or placebo (55). The children were monitored until 5 years of age and there was no difference in the number of asthma diagnoses at the end of the treatment period. The children studied in the PEAK (Prevention of Early Asthma in Kids) trial were older (2–3 yr old) and at very high risk for the development of asthma (based on an asthma predictive index) (56). These children were randomized to treatment with fluticasone or placebo continuously for 2 years, followed by a 1-year observation period to assess the long-term effects of intervention. During the treatment period, the children who received fluticasone had more symptom-free days and fewer wheezy exacerbations, suggesting a good therapeutic response to ICS, similar to other reports (57). Unfortunately, these responses diminished after cessation of treatment, and by the end of the 1-year observation period, there was no difference between the fluticasone- and placebo-treated groups.

Taken together, the above three trials suggest that the use of ICS in the treatment of transient wheezing in infants and very young children does not alter the risk to develop persistent wheezing and asthma. In contrast, older preschool children at very high risk for asthma may benefit from treatment with ICS to decrease wheezing, symptoms, and exacerbations, but the use of ICS as prophylaxis to prevent asthma in asymptomatic at-risk children is not warranted at this time. There is a caveat to this conclusion, however. The definitions of at-risk children in the PAC, PEAK, and IFWIN studies were based primarily on clinical assessments or family history. Would prophylactic ICS

be efficacious in children at risk defined using subclinical measures of inflammation, the target of ICS? In an interesting report by Latzin and coworkers, infants of atopic and/or smoking mothers with elevated exhaled nitric oxide levels in the first 2 months after birth were more likely to develop frequent respiratory symptoms in the first year of life (24). Although early wheezing does not always lead to asthma (58) and the utility of noninvasive markers of inflammation in asthma in children has not been shown conclusively (30–32), the concept of identifying children at risk based on maternal atopy and a biomarker is intriguing. Whether early ICS would prevent the development of asthma in these children requires further investigation.

ICS/LABA as a reliever/controller. The concept of using ICS in combination with an LABA concurrently as a scheduled maintenance controller, and an as-needed controller/reliever, is a departure from the traditional division of these two classes of asthma medications. Patient adherence with prescribed inhalers is a major obstacle in asthma care; substitution of separate ICS and LABA inhalers with a single ICS/LABA combination product has been shown to increase patient compliance (59). The next advance in patient care will be to provide daily therapy using a single inhaler (containing both an ICS and an LABA) to treat both acute and chronic asthma symptoms. Budesonide and formoterol in a single inhaler used as maintenance and reliever therapy have been shown to decrease symptoms and asthma exacerbations when compared with twice the dose of budesonide alone (60). Two large clinical trials in 2006 corroborated these previous studies by showing better lung function, fewer exacerbations, and better asthma control with as-needed extra puffs of budesonide/formoterol combination products (61, 62). These studies suggest that intermittent increases in the total daily dose of ICS at an earlier time point in the setting of worsening asthma control may improve symptoms and prevent asthma exacerbations while allowing lower maintenance doses of ICS. Although there is concern that airway inflammation could "silently" worsen at the lower doses of ICS after a step-down to an ICS/LABA combination, several studies have compared markers of inflammation in sputum and bronchial biopsies and have failed to show an increase in inflammation with this treatment approach (63, 64). The major drawback of therapy with a single controller/reliever inhaler (ICS/LABA) is the assumption that the patient's perception of loss of asthma control correlates with airway inflammation and that, by intermittently increasing (then decreasing) ICS dose, you are adequately maintaining long-term control at a biologic level. However, the empirical observations of fewer exacerbations at a lower total ICS dose would appear to dispute this hypothetical consideration.

Anti-Tumor Necrosis Factor- α As a Potential New Controller Medication

Although reports examining several different innovative therapeutic modalities appeared in the literature this year, clinical trials of anti-tumor necrosis factor (TNF)- α immunomodulators were perhaps the most anticipated. There is a plethora of literature supporting the pathobiologic role of TNF- α in asthma and, most recently, this inflammatory cytokine has been shown to be particularly important in severe refractory asthma (65). An initial small, uncontrolled, open-label clinical trial of etanercept (a soluble TNF- α receptor-IgG fusion protein) in 17 patients with severe asthma reported clinical improvement in asthma symptoms, lung function, and bronchial hyperresponsiveness, which stimulated considerable interest in using anti-TNF- α drugs in this group of patients (66). Two randomized, double-blind clinical trials of TNF- α modulators were published in 2006. A crossover study of 10 weeks of etanercept given subcutaneously in 10 patients with severe asthma confirmed the findings

of the initial study, finding decreased asthma symptoms and bronchial hyperresponsiveness, with increased lung function (67). The second placebo-controlled study of 6 weeks of infliximab (a monoclonal antibody against TNF- α), given as an intravenous infusion in 38 patients with moderate asthma, showed no change in the primary efficacy variable (PEF) (38). This lack of clinical response is particularly troublesome given that LABAs were not allowed in the trial to avoid possible masking of a real change in lung function due to bronchodilator effects of these drugs. In the latter study, there were fewer asthma exacerbations in the treated group, but these were defined as a decrease in PEF on 2 consecutive days or an increased need for as-needed bronchodilators, not by need for urgent care or oral corticosteroids. On the basis of these initial clinical trials, it appears that antagonism of the TNF- α axis may have a role in treatment of severe refractory asthma, but large long-term, placebo-controlled trials will be necessary to evaluate the effect of these drugs on corticosteroid dependency and severe exacerbations, the hallmarks of severe asthma. These trials should attempt to identify the subset of patients who will respond to TNF- α antagonism, because it is likely that a substantial number of patients with asthma will not experience improvement with this intervention. Attempting to identify such a responder subset is not a component of conventional clinical trials; creating paradigms in which such an approach is possible is important, as we attempt to personalize our approach to each individual patient with asthma.

PATHOBIOLOGIC MECHANISMS IN ASTHMA

Gene–Environment Interactions

Although traditional genetic studies continued to look for associations between asthma phenotypes and/or candidate genes in large family (68) and case-control cohorts (69, 70), there was a new emphasis on the interaction of genes and the environment in 2006. These studies focused on the effect of the environment on the translation of previously identified candidate genes to asthma phenotypes, ranging from clinical responses to drugs or treatment to exposure to endotoxin, environmental smoke, ozone, or animals (71–77).

Pharmacogenomics. In a pharmacogenetic analogy, the environment is the drug and the gene is the therapeutic target of that drug. The most studied drug–gene interaction is the ADR β 2 gene and the potential differences in response to β -adrenergic agonists, both SABAs and LABAs, in an individual patient (discussed *above*). Another class of drugs with known genetic variation where gene–environment interactions could explain differences in clinical responses is the leukotriene modifiers. The American Lung Association Asthma Clinical Research Centers evaluated multiple SNPs in genes in the arachidonic acid-lipoxygenase pathway as part of a 6-month randomized trial of montelukast as add-on therapy in patients with poorly controlled persistent asthma (71). There were significant associations between five common SNPs (and one tandem repeat) in the leukotriene pathway, with variable clinical responses to montelukast as measured by change in lung function or exacerbation rates, suggesting a genetic component to a lack of treatment response. The next drug–gene interaction to be intensely studied will likely be the effect of genetic variation in the glucocorticoid receptor pathway on the response to corticosteroids (78). These studies will be crucial, given the mounting evidence for corticosteroid insensitivity (or resistance) in patients with severe asthma (79, 80).

Endotoxin and ozone. The potential gene–environment interaction of a promoter SNP in the CD14 gene (–159 T to C) and exposure to endotoxin is of particular interest given prior reports

of decreased asthma in children exposed to farms (81) and the binding of endotoxin by a well-published candidate gene, CD14 (82). In the European Community Respiratory Health Survey, a self-reported history of childhood exposure to farms decreased the risk of allergy and atopy in adulthood; this effect was most marked in adults with the TT genotype (73). In a birth cohort of 5-year-olds in England, children with the CC genotype had a higher risk for atopy and eczema, but only when exposed to low levels of endotoxin measured in house dust (74). As endotoxin exposure increased, atopy decreased in the children with the CC genotype in a dose-dependent manner. Endotoxin levels had no effect on the expression of allergy-related phenotypes in children with the TT genotype. Although the findings of these two studies may seem disparate, the differences in intensity (low or high) and timing (early in life or later) of endotoxin exposure may contribute to the variation in results. In a similar paradigm of dose-dependent changes in gene expression, school-aged children in the Children's Health Study with the GG phenotype of a previously published SNP in the TNF- α gene (–308 G to A) had an overall decreased risk of asthma, but this protective effect was most marked at low levels of ozone exposure as compared with higher levels (76). These studies illustrate that gene–environment interactions are nonlinear and complex; future studies will need to control for dose and timing of environmental exposures in their analyses.

Remodeling in Asthma

The direction of cell biologic research has also changed in the past year with increasing focus on the structural components of the airway: the epithelium, airway smooth muscle (ASM), and vasculature. There is growing evidence for a major pathophysiologic role of ASM in asthma (83, 84). Bronchial biopsies from patients with asthma have shown ASM hyperplasia and hypertrophy; these cells secrete increased amounts of cytokines and growth factors that recruit inflammatory cells and stimulate production of extracellular matrix (ECM) proteins (85–87). An interesting report from Kaur and colleagues showed increased expression of chemokine receptors on ASM, myofibroblasts, and fibroblasts with migration, not proliferation, of ASM *ex vivo*, suggesting that the increase in number of ASM cells in asthma might be, at least partially, due to recruitment of ASM progenitors from the periphery (88). Sampling of the distal lung via transbronchial biopsy showed differences in the morphology, proliferation, and production of mediators in fibroblasts from the proximal as compared with the distal airways, further complicating our understanding of ASM and fibroblasts in asthma (89). Of interest, in an ovalbumin-induced murine model of asthma, treatment with a leukotriene receptor antagonist, but not corticosteroids, reduced ASM mass and collagen deposition, questioning the ability of ICS to reverse this aspect of airway remodeling (12).

Vascular remodeling. A microvascular component to airway remodeling was shown in several studies documenting increased vascular endothelial growth factor (VEGF) and histologic evidence of angiogenesis in bronchoalveolar lavage fluid, proximal airway biopsies, and sputum from patients with asthma (90–92). Matrix metalloproteinase (MMP)-9, a metalloproteinase that degrades ECM, was also elevated in the sputum from patients with asthma, and MMP-9 levels correlated with VEGF (91). Antagonism of the VEGF receptor in an ovalbumin-induced murine model of asthma down-regulated both VEGF and MMP-9, and reduced the asthma phenotype in these mice (91). In a primate model of asthma, repeated exposure to allergen increased VEGF in the airway wall. This effect was most pronounced in the smaller generation airways, not the proximal airways (8). Such small airways would not be typically sampled

in a human volunteer and it may be that microvascular remodeling is more extensive than previously believed.

Remodeling in children. Although there is increasing evidence for a major pathobiologic role for mesenchymal cells in asthma, there has been debate as to whether airway remodeling occurs as a primary event (cause) or secondary to chronic airway inflammation (effect). Two pediatric airway biopsy studies reported the presence of components of airway remodeling in young children with mild to moderate asthma (92, 93). These findings suggest that airway remodeling is not simply the result of chronicity or severity of disease but rather a significant contributor to the basic pathobiologic mechanisms in asthma.

ADAM33. The gene that best exemplifies the increasing significance of the ECM and mesenchymal cells in asthma pathogenesis is ADAM33 (a disintegrin and metalloproteinase 33). ADAM33 is expressed in ASM, fibroblasts, and myofibroblasts and plays a role in cell signaling, adhesion, and proteolysis (94). The association of ADAM33 with asthma phenotypes as a “susceptibility gene” has been replicated in several cohorts, and genetic variation in this gene has been linked to accelerated decline in lung function (95). In an exciting article this year, Lee and colleagues applied this genetic knowledge to cell biology and measured ADAM33 levels in bronchoalveolar lavage fluid and endobronchial biopsies from subjects with asthma (96). They found a correlation between higher ADAM33 levels and increased chronic airflow obstruction. This would imply that ADAM33 is not just an asthma susceptibility gene but also a biomarker for disease severity, supporting a complex cause-and-effect role for airway remodeling in asthma.

CONCLUSIONS

The past year has seen a number of striking developments in asthma research. We have been sternly cautioned in using LABAs by the FDA, and eagerly await mechanistic insight into the apparent increase in severe events observed in patients with asthma taking these agents. Although pharmacogenetic approaches might shed light into this area, data surrounding the ADR β 2 gene, particularly the significance of the Arg16Arg genotype, have become more confusing. Future clinical studies will require meticulous attention to experimental trial design, including extensive haplotypic analyses of the ADR β 2 gene, to begin to suggest, then test, mechanistic hypotheses by which genetic alterations might lead to adverse events.

The results of the several pediatric intervention trials discussed in this Update had been eagerly awaited. The fact that intervention with ICS did not prevent the emergence of “asthma” in infants and children, although not surprising, was disappointing. Although the search for additions to the asthma therapeutic arsenal proceeds at a steady pace (Will anti-TNF- α approaches to asthma be effective in subsets of patients with asthma we can identify?), the most promising, and potentially paradigm-shifting, approach to asthma therapy involves the use of a now FDA approved ICS/LABA combination of budesonide and formoterol, in a way not now approved for use in the United States: as both a maintenance and reliever therapy. If worldwide experience continues to demonstrate significant benefit/risk advantages for this approach to asthma pharmacotherapy, we ask our industry and regulatory colleagues to work together to make this approach to asthma therapy available in the United States.

What will 2007 bring? We await the release of the new asthma guidelines by the National Heart, Lung, and Blood Institute (NHLBI) and partners that are said to stress patient-centric issues, including impairment and risk. We anticipate further guidance from the ongoing Asthma Phenotypes Task Force (NHLBI, American Academy of Allergy, Asthma and Immunol-

ogy, American Thoracic Society, European Respiratory Society) whose task is to encourage the development and use of consensus definitions of different phenotypes of asthma in clinical trials and mechanistic studies. Taken together, these groups should direct us toward better characterization of patients and assessment of risk, both for medications and for life-threatening events, allowing an individualized approach to health care.

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