Conference Summary

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The Golden Anniversary Meeting of the Thomas L. Petty Aspen Lung Conference, held at the Given Institute from June 4–7, 2008, was devoted to the theme of “Asthma: Insights and Expectations.” A review of the summaries of the first 48 Aspen Lung Conferences published in Tom Petty’s splendid “History of the Aspen Lung Conference” (1) shows that the designated conference summarizers commonly express gratitude and admiration to the Meeting Chairs for assembling outstanding speakers expert on the most urgent and topical issues related to the conference theme, for clustering the presentations logically, and for leading lively, engaging discussion. Such an expression is certainly due again, to Richard Martin, Chair, and to Rand Sutherland and Monica Kraft, Co-Chairs. They put together and led a superb meeting. Another common thought of conference summarizers is a feeling of inadequacy to the task. This was expressed even by as eloquent and masterful a summarizer as the late David Bates at the first “Aspen Emphysema Conference” devoted to asthma, in 1961 (2). No less than the expression of thanks and admiration to the conference Chairs, an admission of a feeling of inadequacy is due again, and is here expressed. Forgivable, I hope, for a conference with twelve State of the Art presentations, 24 oral abstracts, and 17 posters on topics ranging from a novel mechanism of regulation of MUC5AC expression in airway epithelial cells to comparison of different treatments in controlling asthma in large numbers of patients.

An idea put forth by David Bates—which he described as an example of “the tyranny of words” in medicine—was that “asthma” may refer to a common pattern of clinical expression of very different pathophysiologic mechanisms. Jeff Drazen echoed this idea in the Aspen Lung Conference most recently devoted to asthma, in 2002, entitled “Asthma in the New Millennium” (3). His summary was a little unusual in that it had its own title, “Asthma and the Human Genome Project.” Jeff’s focus was on the physiologic abnormality central to asthma—obstruction to airflow—noting the multitude of mechanisms that impact the airway wall. He described the study of asthma as at a crossroad, because of the need for a way to distinguish the relative contributions of each mechanism to the overriding asthma phenotype, with different mechanisms predominating in different subpopulations of individuals with asthma. He proposed that this distinction would be made through dissecting the reasons for differences in the response of different patients to highly specific treatments (e.g., anti-IgE, anti-LT), and that this in turn could lead to the identification of different susceptibility genes. Coming full circle, identification of these genes could lead to simple genetic tests enabling rapid identification of the likely mechanisms responsible for asthma in an individual patient, permitting selection of the therapy most effective for that person. Jeff thus saw asthma treatment as on the threshold of a new era, in which the fruits of the Human Genome

Project would advance understanding, diagnosis, and treatment. At least three talks at this year’s conference followed similar themes. Dirkje Postma updated the state of asthma genetics (*see below*), and Charles Irvin and Julian Solway focused on the consequences and causes of obstruction to airflow. Noting the weakness of the relationships between FEV₁ and radiographic measurements of the diameters of medium and small airways, Charles referred to the idea that FEV₁ faithfully reflects the complex architecture of the lungs and airway caliber as illusory (4). He emphasized the effects of airway closure on lung volumes as important in asthma, and called attention to luminal filling by mucus and by fibrin as causes of the highly heterogeneous pattern of airway closure demonstrated by imaging studies of patients with asthma (5–7). Julian detailed the role of increased smooth muscle mass in narrowing the airway lumen and in increasing bronchial reactivity (8), and highlighted TGF- β as a mediator of smooth muscle hyperplasia and Lovastatin treatment as a possible means of reversing it.

As so often happens in asthma research, pathways that once seemed straightforward as well as promising turn out to be complex, with multiple intersections and redundancies. Other presentations at this year’s conference suggested that other crossroads are critical, as of the pathways of innate and cognate immune responses, of the airway epithelium and cells of the immune system, and of the maturation of immune function and its encounter with the microbial world.

ASTHMA EXPECTATIONS

Although prominent in the conference title, not much attention was paid to “asthma expectations.” Patricia Finn did note that the condition affects 300 million people on our planet, 22 million in the United States, many of them children, and that the morbidity and economic costs of asthma are unacceptably high (9). From speaking not with the conference participants, but with their accompanying friends and spouses (an admittedly highly informed, nonrepresentative sample), it is clear that the lay public ultimately expects the development of a cure for those with asthma and of an effective means of primary prevention for those who do not yet have it. They understand that fulfilling these expectations will require good understanding of the causes and mechanisms of asthma, so they appreciate the need to do research, but they don’t want us to take too long about it.

How does the current state of asthma care and research measure up against these expectations? Rob Lemankse’s masterful “revisiting” of landmark studies of asthma therapies noted that while we have learned much about the best use of the therapies we have, we do not yet have much in the way of new treatments. Our armamentarium consists basically of short-term relievers that may be harmful if taken frequently and long-term controllers that are highly effective in about 70% of patients (10). And even in those patients, they are effective only so long as they are taken continuously, and do not appear alter disease progression (11).

As for prevention, medical science is seen as almost clueless. We know to advise people to avoid exposure of children to second-hand cigarette smoke (12), to breast feed babies for 6 months but maybe not longer (13), to have large families (14), or to take up farming with domestic animals (15). But we don’t seem to know whether to advise buying two dogs or two cats (16), to avoid peanuts or eat them early (17), to send young

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children to day care to ensure they contract multiple viral respiratory infections (18) or to treat them with immune globulin for RSV bronchiolitis (19). This is why the second key word of the conference title, *insights*, is so important, so long as we keep the expectations in mind: to develop a cure and a means of primary prevention.

ASTHMA INSIGHTS

It is in presenting insights into the causes and mechanisms of disease that the Aspen Conference is strongest.

AIRWAY EPITHELIUM

Yvonne Janssen-Heininger opened the presentations by calling attention to the importance of the airway epithelium in asthma's pathogenesis, singling out NF- κ B as a key transcription factor at the crossroads between innate and adaptive immunity (20, 21). She showed data from animal models that selective activation of the canonical NF- κ B pathway in the airway epithelium causes neutrophilic inflammation, smooth muscle thickening, and airway hyperreactivity in the absence of administration of antigen or Th2 cytokines, and without inducing mucus metaplasia or eosinophilic infiltration (20). She also presented a provocative perspective on the existence of a noncanonical pathway to activation of NF- κ B and to activation of genes important in the adaptive immune response. While these pathways can be reciprocally inhibitory, their interactions may be more complex, as we have learned of Th1 and Th2 lymphocyte responses (22). Patricia Finn continued the theme of interaction between innate and adaptive response mechanisms, reviewing pathways by which innate immune responses regulate adaptive immunity, initiated differently by activation of different Toll-like receptors in innate immune cells, including cells of the airway epithelium (23). Bob Schleimer's review of regulation of inflammation and immunity by epithelium dovetailed nicely. Using tissues from patients with chronic rhinosinusitis to study epithelial function in inflammatory diseases, he showed innate responses to have important regulatory effects on dendritic cell, T cell, and B cell function. He introduced the possible importance of infection in shaping epithelial cell function by reporting that infection with rhinovirus caused airway epithelial cell cultures to produce thymic stromal lymphopoietin (TSLP), a possibly key initiator of allergic inflammation, synergistically amplified by IL-4 addition at the time of RV infection (24). He further showed that RV infection of epithelial cell cultures induces production and secretion of B cell activation factor (BAFF) and of a proliferation-inducing ligand (APRIL). He proposed further that BAFF might drive a local immunoglobulin response by B cells in chronic rhinosinusitis as well as following acute infection with RV or other activators of TLR3. Still more evidence of the epithelium's importance came, somewhat unexpectedly, from Qutayba Hamid's discussion of whether airway remodeling is real. He showed that it is, and that epithelial cells might be important in its causation (25). He has found that bronchial biopsies from individuals with severe asthma produce higher levels of IL-8, IL-17A, and IL-17F, and that fibroblasts co-cultured with epithelial cells from subjects with asthma generate the extracellular matrix found in subepithelial fibrosis.

Dirkje Postma's elegant review of the genetics of asthma was in keeping with Jeff Drazen's predictions, and reinforced focusing on the airway epithelium's role in asthma pathogenesis. First, she reported confirmation of linkage of a G protein-coupled receptor (GPCR) gene with asthma (26, 27). This gene, "GPRA" (for G protein-coupled receptor for asthma suscepti-

bility), exists in A and B isoforms. The B isoform is consistently found to be overexpressed in the airway epithelium of patients with asthma. Dirkje additionally reported that genetic pathway analysis shows asthma to resemble inflammatory bowel disease, in which overlapping loci point to T cell signaling and gut barrier function, a function fundamentally dependent on gut epithelium.

John Fahy's report of his studies of bronchial epithelial cells from patients with asthma continued this theme of the meeting, and harkened back to David Bates' allusion to the possible existence of subtypes of asthma. John studied the pattern of gene expression in airway epithelial cells obtained from patients with asthma, patients with COPD, and healthy control subjects, with the individuals with asthma being re-studied a week after double-blind treatment with an inhaled corticosteroid or placebo (28). He found that the genes most highly overexpressed in the epithelial cells of the individuals with asthma were CLCA1, serpin B2, and periostin, genes that are up-regulated on addition of IL-13 to cultured airway epithelial cells *in vitro*. This was not true of all the subjects with asthma, however, inviting the question as to whether there may be distinct molecular phenotypes of asthma, and whether these phenotypes differ in their responsiveness to ICS therapy.

VIRAL INFECTION

Most respiratory viruses first—if not solely—infect the airway epithelium, and release of cytokines, chemokines, and other mediators from airway epithelial cells is thought to initiate the cascades responsible for virus-induced bronchitis and exacerbations of asthma and COPD (29). In introducing his comments on "Bugs and Asthma: A Different Disease?" Stokes Peebles noted that virus-induced wheezing illnesses in the first years of life often precede the development of asthma, and showed data from Rob Lemanske and coworkers' "COAST" study suggesting that the risk of having asthma at age 6 years is related to the specific viral pathogens isolated (30). Wheezing illness from a rhinovirus infection was by far the most strongly associated with asthma diagnosis in childhood (Figure 1).

This finding invites the question as to what determines the severity of virus-induced illness: environment or genetics? Stokes noted the importance of considering the genetic variations not just among the people infected, but also among the infecting strains of virus. His work analyzed the determinants of response to respiratory syncytial virus (RSV), for which severe infection in childhood has also been linked to the development of asthma. Unlike rhinovirus, RSV infects mice, so it lends itself well to detailed study in an animal model. Stokes' work has shown that different strains of RSV differ in their induction of bronchial epithelial IL-13 expression and mucus production, and in induction of airway hyperreactivity (31). He has even been able to identify the region of the RSV Line 19 strain necessary for inducing these changes. It is thus plausible that genetic differences among species of rhinovirus—a highly diverse genus of the *Picornaviridae* family—might also differ in their "asthmagenicity."

BEGINNINGS OF ASTHMA

If infection with certain, particularly "asthmagenic" strains of respiratory viruses is important in the causation of asthma, the infection most likely occurs in infancy or early childhood, for—as Fernando Martinez reminded us in his review of "The Beginnings of Asthma"—the origins of the great majority of cases can be traced to first three years of life, possibly even to the prenatal period. Fernando emphasized that asthma is a developmental disease, noting that "persistent wheezers"—children with wheezing in association with respiratory tract infections in first 3

years—had the highest rate of asthma at age 6 years and the poorest pulmonary function through puberty. He noted, too, that bronchial hyperresponsiveness is already established in the preschool years and influences the expression of asthma phenotypes up to early adult life, and that the coincidence of early wheezing and atopy is associated with worst asthma outcomes (32). Emphasizing the importance of events in early life, Fernando noted that few who had not been “persistent wheezers” in early childhood developed asthma by age 22. Events in early life might equally be protective against the development of asthma, as Fernando noted in alluding to epidemiologic data, much of it collected by von Mutius and her collaborators (15), that prenatal and postnatal exposure to domestic animals is associated with striking reductions in childhood rates of allergy and asthma.

Erwin Gelfand picked up this theme in his talk on “Pediatric Asthma: A Different Disease?” He started from the premise that if we are to have any impact on the natural history of asthma, we must intervene during the early years, before the disease is established. With that in mind, Erwin reviewed studies of the response to RSV infection and a subsequent reinfection, in mice of different ages, noting that the age at initial infection dictated the response to the subsequent RSV infection (33). In neonatal mice, this sequence of infection induced an asthma-like phenotype—very similar to the response to allergen sensitization and challenge—with enhanced airway reactivity, airway eosinophilia, and mucus hyperproduction. By contrast, this sequence of RSV infection in adult mice did not induce pulmonary eosinophilia, alter airway function, or increase mucus production.

INTERIM SUMMARY

Taken together, these several presentations at this Aspen Lung conference suggested the following:

1. That viral respiratory infections causing wheezing in the first 3 years of life are associated with a heightened risk for the development of asthma by age 6.
2. That the neonatal (and probably prenatal) and early childhood environment influences the risk of development of allergy and asthma.
3. That the airway epithelium
 - a. is the site of infection by respiratory viruses.
 - b. is at the crossroads of innate and adaptive immune function.
 - c. is a rich source of proinflammatory cytokines and chemokines.
 - d. behaves differently, as if more activated, in people with asthma.
4. That the response to infection with RSV
 - a. is dependent on the age of the animal infected.
 - b. is dependent on the strain of RSV inoculated.

PREVENTING ASTHMA

Slightly paraphrasing the challenge laid down by Erwin Gelfand, the best way to have a real impact on asthma would be to develop a strategy for asthma prevention, before the disease is established. The tentative conclusions listed above suggest a possible approach to developing such a strategy. One of the key elements of the rationale is the belief that infection with an “asthmagenic” strain of a respiratory virus, particularly a rhinovirus, is involved in the induction of asthma in susceptible infants or young children. Another element is the belief that

whether infection with an “asthmagenic” strain of a respiratory virus induces asthma is partly a function of the effectiveness of immune function at the time of infection. And a third element is the idea that the effectiveness of immune function increases during infancy, at a rate that can be affected by microbial exposure in infancy, especially at epithelial surfaces.

SEVERITY OF RESPIRATORY TRACT INFECTIONS

The properties that confer “asthmagenicity”—if there is indeed such a property—on a strain of a respiratory virus are not known. What has recently become established, though, is that there is great diversity among rhinoviruses. A previously unrecognized phylogenetic branch of the genus, Human Rhinovirus “group C,” has been discovered (34, 35). Members of this group are as different from the known HRV groups A and B as they are from each other. They are speculated to cause more severe clinical disease, but little is known about their function, since no member of the group has so far been cultured.

The severity of illness caused by infection with a respiratory virus is of course a function not just of the virus’s properties but also of the effectiveness of the host’s antiviral defense. This defense is at least in part mediated by Th1 cytokines, for they have been shown to be indispensable for effective cell-mediated immunity, which is necessary for eradication of intracellular pathogens, including viruses (36). That this is true of viral respiratory infections in human infants is supported by the observations that infants who produce high levels of Th1 cytokines to RSV infection have a milder clinical course (37) and that infants whose cord blood PBMCs produce low levels of IFN- γ in response to PHA stimulation have more viral illnesses in their first year of life (38).

The relevance of these observations to the pathogenesis of allergic disease, including asthma, is highlighted by our knowledge that human infants are born with a “Th2 bias” and that development of robust cell-mediated immunity requires production of Th1 cytokines (39). It further appears that in infants at risk for development of atopy, Th2 polarization is more prominent and lasts until an older age (40–42).

CAN ANTIVIRAL DEFENSE BE MODIFIED? THE HYGIENE HYPOTHESIS REVISITED

It is clear that antiviral defense develops over infancy. That is why the administration of live attenuated influenza vaccine is delayed until the age of 5 or 6 months (43). Whether the rate of maturation of antiviral defense is modifiable is much less well established. But if infection with certain viruses in infancy is important in the pathogenesis of asthma, then a growing body of epidemiologic data can be viewed as supporting the idea that the burden or nature of microbial exposures in infancy might accelerate such maturation. Exposure from birth to the microbes inevitable in households with children might account for Strachan’s landmark observation that the risk of asthma and allergy is greater in first-born children than in younger siblings (14). Exposure to certain types or numbers of microbes in infancy might similarly account for the protective effects from growing up in households with two or more dogs (16), or on a farm with domestic animals (15), or from drinking unpasteurized “farm milk” (44).

The idea that development of immune function might be modifiable in human infants is indirect, coming largely from epidemiologic studies. These studies appear to point to microbial flora in the gastrointestinal tract as the site where modification takes place. Early studies showed differences in stool flora of infants raised in areas with high and low prevalence rates of asthma. *Lactobacilli* and bifidobacteria predominated in

Figure 1. Virus-Induced wheezing in infancy and development of asthma. Risk of asthma at age 6 years in children without a wheezing illness ("neither") versus those who wheezed during infection with respiratory syncytial virus, rhinovirus, or both (* $P < 0.05$ versus neither, + $P < 0.05$ versus RSV only). Adapted by permission from Reference 30.

the low-prevalence areas (45). More impressively, a prospective birth cohort study of nearly 1,000 infants examined relationships between bacteria detected in stool samples of 1-month-old infants and their subsequent development of atopic manifestations and sensitization 2 years later. The presence of *Escherichia coli* was found to be associated in a dose-dependent manner with a heightened risk of developing eczema. Colonization with *Clostridium difficile* was associated with heightened risks for eczema, recurrent wheeze, and allergic sensitization (46).

Direct evidence of a relationship between intestinal microbial community structure and antiviral immune function comes from a study of influenza infection in BALB/c mice (47). Compared with control littermates, mice pups fed *Lactobacillus casei* supplement for 3 weeks before inoculation with influenza virus had better survival (40% versus 14%), lower viral titers in nasal lavage fluid, higher pulmonary NK cell activity, and greater IL-12 production by mediastinal lymph node cells.

A STRATEGY FOR ASTHMA PREVENTION

These observations provide enough insight into the causes and mechanisms of asthma to propose a rationale for meeting one of the expectations of the scientific study of asthma, development of strategy for primary prevention of asthma, if not for development of a cure. The elements of the rationale are as follows:

1. Infection with some strains of common respiratory viruses early in life predisposes to the development of asthma.
2. The consequence of infection with these viruses is a function of the effectiveness of antiviral immune function at the time of infection.
3. Antiviral immune function increases in efficacy throughout infancy.
4. The rate of development of antiviral immune function is influenced by the composition of gut microbial flora.
5. The composition of gut microbial flora is influenced by diet and by the external microbial environment.

The essence of the strategy supported by this rationale is to intervene in infants at risk for asthma by shortening the period over which they are at risk of infection by an "asthmagenic" strain of respiratory virus—by shortening the "induction phase" depicted in the figure Erwin Gelfand presented (Figure 2). This may be achievable by direct feeding of microbes that foster maturation of immune function ("probiotics") or by feeding nutrients favoring growth of such microbes in the gut ("prebiotics").

Figure 2. Conceptual model of the "stages" of asthma, emphasizing a period in early life of vulnerability to the induction of asthma by microbial infection and/or by allergen exposure, as distinct from a later "maintenance phase" in which existing asthma is aggravated by exposure to certain allergens, microbes, or atmosphere pollutants.

This strategy may be invalid or unfeasible. But that it can even be proposed calls attention to the possibility of fulfilling the expectations of the kind of scientific research reported at this and other Aspen Conferences. My own view is that the key insights will come from greater integration of the implications of epidemiologic and clinical research into animal research. They will come from the application of new, unbiased culture-independent methods for searching for infection in clinical samples, especially from infants, from detailed characterization of immune function at time of infection, and from equally detailed characterization of the strains of the microbes identified. They will come from animal studies of the relationships between age, diet, environmental exposures, immune function, and responses to viral infection and to allergen sensitization and challenge. They will come from the development and testing of methods to enhance or retard antimicrobial immune function. And they will come from study of the relationships of environment to gut flora, airway flora, immune function, and risks of allergy and asthma.

Admittedly a tall order. But judging from the breadth, depth, and quality of the science presented at this Golden Anniversary of the Aspen Lung Conference, it is one that will be filled.

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