Chapter 1
Introduction to Asthma and Phenotyping

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Keywords Asthma • Heterogeneity • Clinical phenotypes • Clusters • Physiology • Treatment • Molecular phenotype • Induced phenotype

1.1 Introduction and Primer on Asthma and Its Heterogeneity

Asthma is a heterogeneous disease process that is characterized by three cardinal features: (1) chronic inflammation of the airways leading to (2) variable airflow obstruction and (3) airway hyperresponsiveness (NHLBI 2007; Balzar et al. 2011). Over the past two decades, there has been a rise in asthma prevalence, such that about 8 % of the population in 2010 and more than 25 million Americans are affected (Akinbami et al. 2012). The clinical presentation varies in degree of severity, but common symptoms include wheezing, shortness of breath, and cough (NHLBI 2007). There has been a steady increase in asthma prevalence from 2001 to 2010 which is particularly marked in the pediatric population; children have required increased emergency room visits and hospitalizations (Akinbami et al. 2012). Despite the overwhelming prevalence of asthma worldwide, extensive healthcare costs and immense economic burden, a detailed understanding of the underlying pathophysiology of asthma, particularly those features leading to variable expression of disease (recognized as clinical phenotypes) remains to be

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A.R. Brasier (ed.), *Heterogeneity in Asthma*, Advances in Experimental Medicine and Biology 795, DOI 10.1007/978-1-4614-8603-9_1,

developed (Bousquet and Khaltaev 2007). Asthma affects industrialized nations as well underdeveloped countries, and similar phenotypes have been recognized across these borders (Weiss et al. 1992). Recent investigations have noted distinct clinical asthma phenotypes, but the characteristics that separate these phenotypes overlap significantly, including those factors that contribute to the difficulty in clinical management of these patients (Calhoun et al. 2003).

As noted elsewhere (Chap. 6), current management of asthma rests on short-acting beta-2 bronchodilators, inhaled corticosteroids, long-acting bronchodilators, and a small number of other agents. Heterogeneity in response to therapy is a key management issue, reviewed in Chap. 8. The heterogeneity of asthma extends beyond the clinical symptoms and response to therapy, to variable ages of onset, duration of disease process, extent of airway obstruction (NHLBI 2007), nature and sensitivity to triggering agents, characteristics of the airway inflammatory process, and perhaps even the fundamental nature of the immune response.

Clinicians and researchers have used a variety of approaches to categorize, to understand, and ultimately to utilize heterogeneity of expression of asthma to inform clinical management, improve patient care, and strengthen experimental designs. To date, most of these approaches have had limited penetration because of invasive testing (bronchoscopy), overlap among identified groups, and lack of predictive power. In fact, empiric therapeutic trials of various agents, guided by the USA and other international guidelines (NHLBI 2007; GINA 2011), are the norm for clinical care of asthma patients because predictive testing is not available.

Accordingly, classifying a heterogeneous disease like asthma into several smaller, more homogeneous subgroups ("phenotypes") offers the possibility of clearer delineation of asthma mechanisms; more effective, mechanism-based therapy; and improved prediction of disease course.

1.2 Phenotyping Strategies

Phenotypes are groups of patients that are definable based on observable characteristics. In this brief review, we will focus on two complementary phenotyping strategies: clinical phenotyping and molecular phenotyping, with brief mention of cellular phenotyping. Although there are several approaches to asthma phenotyping in the literature, clinical phenotyping and molecular phenotyping remain the two most productive approaches (Wenzel 2012). The goals of phenotyping are multifold, including forecasting the clinical course, predicting response to therapy, identifying subgroups at risk for adverse events and other complications, and reducing clinical heterogeneity in clinical trials (Pillai et al. 2012).

Clinical phenotypes have been defined in asthma for decades: intrinsic (i.e., non-allergic) vs. extrinsic (i.e., allergic) asthma is one such example. Clinical phenotypes attempt to provide reproducible groupings of patients based observed signs and symptoms, combined with testing carried out as part of usual clinical care, and as such is most commonly an observational approach. Clinical phenotyping focuses

on the observed clinical presentation including response to therapy, known inherent characteristics such as weight and sex, and outcomes of diagnostics tests to classify patients into subgroups (Pillai et al. 2012). In the clinical arena, a patient's "phenotype" describes prominent observed characteristics that arise from gene expression and, importantly, can be influenced by environmental elements. Several environmental factors can influence the phenotype including noninfectious allergens, infection mediators such as viruses, and medications that are often chronically administered (Wenzel 2004). These latter phenotypes, dependent on a specific environmental interaction, can legitimately be considered to be induced phenotypes (vide infra).

In contrast, molecular phenotyping looks beyond the visualized presentation and categorizes patients at a molecular level with an emphasis on the genetic makeup, inflammatory pathways, and signaling mechanisms (Pillai et al. 2012; Wenzel et al. 1999). Using these approaches, researchers have made much progress in understanding the mechanistic pathways in asthma that may govern the expression of asthma in heterogeneous groups of patients. Molecular phenotyping attempts to produce reproducible groups based on a molecular signal or signature, which may be protein based, mRNA based, DNA based, or conceivably metabolome based. Fundamental to understanding molecular phenotyping are the concepts that molecular methodologies may provide information that enhances the understanding of pathogenic pathways and that no single measurement or marker is likely to provide sufficient discriminant function to classify unambiguously a heterogeneous group of patients. Hence, many molecular phenotyping approaches employ panels, or groups of analytes, to enhance the performance characteristics of the approach.

In the last decade, technological advances have supported asthma research in both the clinical and molecular arena in an attempt to combine both these phenotype approaches to optimize asthma management and contribute to the field of personalized medicine (Wenzel 2012).

1.3 Application of Clinical Asthma Phenotypes

Clinical phenotypes use features that clinicians are able to observe. Patients with common characteristics are grouped together in an attempt to guide therapy and management. Asthma is a syndromic disease resulting from airway inflammation, a largely unmeasured feature; hence, phenotyping on the basis of clinical characteristics is hampered by the lack of a "gold standard" against which to test the phenotype. Further, the borders between adjoining subgroups of asthma overlap, reducing the discriminant value of the approach (Busse et al. 1993; Horwitz and Busse 1995). Despite these limitations, there are several prominent clinical phenotypes that are noteworthy, which have assisted clinicians over the decades, including the concept of allergic vs. nonallergic asthma, the syndrome of exercise-induced bronchospasm, and the association of difficult-to-control asthma with obesity and with smoking.

When patients with asthma and positive IgE reactions to commonly encountered aeroallergens are evaluated in the clinic setting, the descriptive term allergic asthma is often utilized. This term is more than shorthand, as allergic asthma is well described and has assisted clinicians over the years in recognizing and managing patients who have exaggerated responses to various stimuli. Moreover, the term "allergic asthma" became commonly recognized in lay society and so has external validity as well. Finally, the term conveyed an understanding of the putative etiology of symptoms for these patients (vide infra).

Exercise-induced bronchospasm is another entity that is well known to practitioners and has distinct features in clinical presentation and therapeutic management that sets it apart. Although exercise can be a trigger for many kinds of asthma, the entity of exercise-induced bronchospasm (EIB) per se occurs solely in the context of exercise, and not in other situations (nocturnal, seasonal, etc.), and the timing of onset of symptoms following cessation of exercise is characteristic. Its management generally relies on prophylactic use of inhaled short-acting beta-agonists prior to onset of exercise, as the degree of airway inflammation in EIB is generally less than that seen in other forms of the disease. Recognizing this clinical phenotype then is helpful to the clinician in formulating therapeutic management plans.

1.3.1 US Severe Asthma Research Program

More recently the US Severe Asthma Research Program (SARP) has analyzed an extensive dataset of patients with severe and non-severe asthma, in an attempt to identify and describe robust subgroups of asthma patients with distinct features (Jarjour et al. 2012), which possibly could be, but as yet has not been shown to be, an informative guide to personalized therapy. The approach used is termed clustering and by use of a variety of statistical analyses interrogates the dataset to identify characteristics that most accurately distinguish among subgroups within the study population.

The prevalence of asthma and health-care costs from asthma-related complications continues to grow despite the aggressive research in many academic centers (Weiss et al. 1992). Hence, the National Heart, Lung, and Blood Institute (NHLBI) established the Severe Asthma Research Program (SARP) made up of eight academic institutions to further investigate this group of patients (Jarjour et al. 2012). SARP has provided much clinical advancement in the field of asthma research. Specifically, in the area of clinical phenotyping, analysis of the SARP population, that incorporated more than 1,500 asthma patients, more than 500 of whom were severe, led to the identification of five "clusters," or clinical phenotypes (Walker et al. 1992). The clustering approach did not presuppose that any particular metric would be predictive. This paper was one of the first to apply the methodology to a large dataset of asthma patients with a wide range of severity.

SARP defined severe asthma as fulfilling at least one major criterion (daily use of high-dose inhaled corticosteroids or use of systemic corticosteroids) and at least two

of seven minor criteria proposed from the ATS guidelines on refractory asthma (ATS 2000). Patients in this study had extensive biologic and physiologic characterization and were followed for approximately 2 years. The five discrete subgroups of asthma patients emerged by agglomerative cluster analysis. Cluster 1, the mild allergic asthma cluster, was characterized by history of atopy and an early onset of the disease. It is interesting to note that these patients had essentially normal lung function and doctor's visits were minimal. Cluster 2 is a prominent group with the largest population with mild-to-moderate allergic asthma. This group like cluster 1 had history of atopy and early onset; however, strikingly this group had borderline low FEV1 values. The next group, cluster 3, was quite different, with an older age range, late onset symptoms, and higher BMI. Cluster 3 patients had a reduced frequency of atopy, but still required frequent systemic corticosteroids and greater than three controller medications. Clusters 4 and 5 had an increased duration of illness and high healthcare utilization and comprised together a third of the patients. Cluster 4, severe variable allergic asthma, had longer duration symptoms, minimal reduction in FEV1, and almost normal reversibility with bronchodilators. In contrast, cluster 5 patients showed more marked reduction in FEV1 and less marked bronchodilator response. Cluster 5 is characterized by fixed airway obstruction which was associated with increased utilization of health-care resources (Jarjour et al. 2012).

1.3.2 Allergic Asthma

As noted, the term "allergic asthma" is used by many clinicians and patients because the term, and associated clinical phenotype, provides physicians with a basic mechanistic understanding of the disease and gives patients an understanding of the cause of their symptoms. Allergic reactions to otherwise innocuous agents remain the hallmark of allergic asthma. These responses are dependent on initial exposure to allergen and subsequent development of IgE antibody that reacts to that allergen, a process known as sensitization. IgE is bound to mucosal mast cells that express the high-affinity receptor for IgE, (FcεR1). Later exposure of those mast cells to the sensitizing allergen results in mast cell activation, inflammatory mediator and cytokine release, and the development of characteristic eosinophilic airway inflammation and airway hyperresponsiveness (NHLBI 2008). Allergic asthma patients thus exhibit airway eosinophilia, mast cell recruitment, positive skin test responses to aeroallergens, and elevated serum immunoglobulin E (IgE) (NHLBI 2008; Abbas et al. 1996; Walker et al. 1992). The concept of Th1–Th2 lymphocyte imbalance is now decades old, and the evidence for Th2 bias in the development of allergic asthma is compelling (Abbas et al. 1996). T-cell differentiation to the Th2 cellular pathway leads to the production of prototypical cytokines, specifically interleukin 4 (IL-4), interleukin 5 (IL-5), and interleukin 13 (IL-13) (Wills-Karp et al. 1998). The role of IL-13 in airway hyperresponsiveness appears to be of particular note, because there are mechanistic, diagnostic, and potential therapeutic implications of this finding (Gauvreau et al. 2011).

1.3.3 Exercise-Induced Asthma

Exercise-induced bronchospasm (EIB) refers to airway constriction that occurs following exercise. The specific underlying mechanisms remain controversial (McFadden 1995). Asthma symptoms, commonly, shortness of breath and wheezing, occur well after the onset of exercise (McFadden 1995) and typically are experienced upon cessation of exercise for up to 10 min after exercise. Recovery time varies, but most patients return to baseline respiratory status within 60 min of exercise cessation (Edmunds et al. 1978). There are two prominent theories underlying the mechanism of EIB: heat loss and hyperosmolality. During and after exercise, the airway mucosa is exposed to rapid, large volumes of air which may be inadequately warmed, not fully humidified, or both (Randolph 1997; Anderson and Daviskas 2000). Either heat loss or water loss (and osmotic change) is thought to trigger release of inflammatory mediators (McFadden and Gilbert 1999; Anderson and Daviskas 1999). Regardless of the initiating mechanism, the clinical presentation of EIB is well described, and aggravation of bronchospasm may be seen in almost half of asthma patients (Hallstrand et al. 2002). This clinical phenotype is useful, because therapeutic use of short-acting beta2 agonists prophylactically prior to exercise can blunt or eliminate the development of symptoms.

1.3.4 Induced Phenotype

The abovementioned clinical phenotypes in asthma give the physicians and researchers a basic structure to categorize patients to understand the disease process and improve clinical management. Infections with bacteria (sinusitis) and viruses and other allergic or environmental exposures can also increase the severity of presentation. In contrast, the administration of effective controller medications can reduce the apparent severity of disease presentation, reducing symptoms, inflammation, and exacerbations. Inhaled corticosteroids remain the mainstay of chronic therapy for asthma; however, relative corticosteroid insensitivity has been noted, particularly in severe disease, and response to ICS is inconsistent (Bhavsar et al. 2008). One can consider a phenotype that is dependent on a specific environmental factor (allergen, virus, controller medication) to be an "induced phenotype": that is, a phenotype not expressed unless the environmental factor is present. This "induced phenotype" is important to recognize as it represents the clinical phenotype in the presence of therapy or other modifying factors (Pillai et al. 2012). The degree to which the clinical-induced phenotypes may inform mechanistic understanding and predict therapeutic responses is not currently known, but is an area of active research at present.

1.4 Cellular Phenotyping

The type and quantity of inflammatory cells in the airway of asthma has been used as a means to categorize the disease. Cellular phenotypes in asthma have been correlated with important features of clinical presentation, with neutrophilic disease being associated with acute severe asthma (Sur et al. 1993), and with steroid resistant asthma (Wenzel). These differences in cellular phenotype may also be associated with altered responsiveness to other therapeutic agents (Fahy et al. 1997; Pearce et al. 1999). Hence, it is plausible to suggest that the nature of cellular inflammation asthma might predict other important clinical outcomes.

Bousquet et al. (1990) demonstrated a correlation with the presence of eosinophils in blood and BAL fluid with asthma severity. Later, Wenzel et al. identified eosinophilic and noneosinophilic cellular subtypes in a group of severe asthma patients and noted a thickening of the subepithelial layer in the eosinophilic phenotype (Wenzel et al. 1997, 1999). The concept of a neutrophilic cellular phenotype was supported by studies that associated increased airway obstruction with neutrophilic infiltration (Shaw et al. 2007; Green et al. 2002). The eosinophilic phenotype is associated with a significant benefit from corticosteroids (Berry et al. 2007), while the neutrophilic phenotype is associated with a poor steroid response (Berry et al. 2007). The neutrophilic phenotype associates with acute severe asthma exacerbations in fatal and non-fatal status asthmaticus (Fahy et al. 1995; Sur et al. 1993). However, cellular phenotypes overlap, and a mixed eosinophilic and neutrophilic phenotype has been associated with severe respiratory symptoms (Hastie et al. 2010).

1.5 Molecular Phenotyping

Identifying, analyzing, and validating the presumed links between specific clinical presentations of asthma ("clinical phenotypes") and the associated underlying molecular pathways may offer further insight in tailoring therapy to a specific patient or patient populations. In essence, uncovering the fundamental mechanisms and pathways will establish the basis of variable clinical presentations. The term molecular phenotyping implies the use of quantitation of specific molecular species to develop mechanistically similar subgroups of asthma. Molecules of interest and potential utility include nucleic acids, proteins, posttranslational modifications, and metabolites. Small molecules are easily analyzed using high-throughput analytics and are therefore attractive candidates for the development of biomarkers. We will review briefly two approaches to molecular phenotyping: gene-expression-based and protein-expression-based methods. Because genetics are essentially static throughout life, genetic associations in genomic DNA may inform presentation and course of disease, response to therapy, and associated clinical features, but do not

well address the clinical temporal variability of asthma, which is punctuated by exacerbation and remissions. To address these questions, an understanding of the regulated elements (mRNA, gene expression; protein types and quantities, protein expression; and end products, metabolites) is necessary. The field of metabolomics in asthma is in its infancy; this review will focus on gene- and protein-expression-based approaches. These brief considerations are exemplary, not exhaustive in scope. A more comprehensive discussion of Genetics, Genomics, and Epigenetics can be found in Sect. 2 (Chaps. 9, 10, 11).

1.5.1 Molecular Phenotyping Based on Gene Expression

In the last decade technological advances have permitted addressing scientific questions regarding the relationship between gene expression and clinical presentation of asthma. A seminal example of this approach was that of Woodruff and colleagues who studied gene-expression profiling of airway epithelial cells in asthma. They identified a group of genes the expression of which was enhanced by IL-13 (Woodruff et al. 2007, 2009). Further, there was variability in IL-13 expression, and subgroups of asthmatics were identified with both high and low levels of IL-13, indicating high and low levels of activation of Th2-like pathways, despite similar symptoms. CLCA1, periostin, and serpinB2 are downstream of IL-13 and were increased by exposure to IL-13. Corticosteroid treatment suppressed expression of these genes (Woodruff et al. 2009). Furthermore, the classification of asthmatic patients into "Th2 high" and "Th2 low" correlated with different degrees of airway inflammation and with corticosteroid responsiveness (Woodruff et al. 2007, 2009). Hence, this approach to molecular phenotyping provided both important mechanistic information and also a potential predictive analytic for inhaled steroid responsiveness.

1.5.2 Molecular Phenotyping Based on Protein Expression

An alternative and complementary approach to molecular phenotyping is based on the expression of proteins in the organ of interest, in this case the airway. Using the sample bank from the US Severe Asthma Research Program, Brasier and colleagues measured a panel of cytokines in BAL fluid from asthma patients with severe asthma and compared them with asthma patients with mild-to-moderate disease (Brasier et al. 2008). Protein expression of a broad panel of inducible proteins was measured, including markers of innate immune activation, Th1 activation, Th2 activation, and relevant chemokines. Using unsupervised cluster analysis, four cytokine expression clusters were identified, one of which was highly enriched with patients who had severe asthma. Further analysis of this protein-expression dataset demonstrated that clinically important intermediate phenotypes could be predicted

solely by protein-expression data and that these intermediate phenotypes in turn mapped to the clinical syndromes of severe asthma and mild-to-moderate asthma (Brasier et al. 2010). These findings suggest that protein-expression data may be a useful platform from which predictive biomarkers for therapeutic response and other clinically important outcomes could be based.

Additional advanced analytics yielded additional insights. Using network analysis and visual analytics, Bhavnani and colleagues examined this same protein-expression dataset (Bhavnani et al. 2011). Cytokine expression clustered by pathway: Th2-like cytokines clustered together, and innate immunity cytokines clutered together, but in a different space than the Th2 cytokines. Further, patients with mild-to-moderate asthma tended to cluster with the Th2 cytokines, whereas the patients with severe asthma tended to cluster with markers of innate immune activation. This analysis then provided important mechanistic insights in both severe and non-severe asthma. Accordingly, the promise of molecular phenotyping for a variety of important predictive analytics is great.

1.6 Summary

Asthma is an inflammatory disorder characterized by airway obstruction, airway hyperresponsiveness, and airway inflammation, all of which are variable among patients and variable in time within any specific patient. Understanding the mechanism that underlies this observed variability, and using that understanding to advance the science of asthma and the care of asthmatic patients, is an essential purpose of developing phenotypes. Clinical phenotypes have been used for decades, but overlap each other, and do not map cleanly to either pathophysiologic mechanism or with therapeutic response. Molecular phenotyping, although as yet only partially developed, offers the promise of dissecting the mechanistic underpinnings of the variability of asthma and of providing predictive therapeutics for the benefit of patients with this common and troubling disease.

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