

EDITORIAL

Clinical & Experimental Allergy

Complex asthma endotypes, differential chemokine responses and birth cohort studies: solving equations with multiple variables

This editorial discusses the findings of the paper in this issue by Følsgaard et al. [1], pp. 1596–1603

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It is increasingly common that ultra-high impact journals, and we as reviewers, require colleagues with groundbreaking discoveries in human research to repeat key experiments in mice to have the work published. The intent of course is to distinguish causality from association, an important goal. The problem is that the model(s) generally utilized, while superbly amenable to manipulation and experimental control, may only weakly reflect the many physiological processes – and the inherent diversity – present in any human population.

An alternative strategy, less efficient for assessing causation but offering a more compelling approach to incorporate the variability introduced by human biology, genetics, environmental interactions and time, is that of studying longitudinal cohorts. Well-powered studies of human populations (general population cohorts, high-risk cohorts, interventional cohorts, etc.), especially those beginning at birth which allow nature to take its course and disease to develop in some proportion of individuals while health persists in others, offer an increasingly utilized approach to identify and integrate these factors. Typically highly multidisciplinary, such programmes bring together expertise to address an outcome (e.g. asthma, cardiovascular disease, dementia), which in reality is usually composed of multiple subtly different outcomes arising via independent mechanisms.

There is widespread acceptance that the first year of life is pivotal in shaping many long-term immune, physiological and clinical outcomes. In this issue, Følsgaard et al. [1] investigate putative relationships between chemokine levels at birth (in cord blood), subsequent development of commonly used surrogate markers of allergic disorders and expression of clinical phenotypes at multiple time-points over the first 6 years of life. This is an innovative approach because, while a few other groups have employed this strategy, only approximately 0.3% of almost 250 000 cohort study derived publications utilized differential chemokine(s) expression as a study parameter.

Chemokines as indicators of immune status

Why chemokines? They offer excellent potential as tools for analysis of immune and immunoregulatory status. For over a decade, we have known that many chemokines and classical cytokines (e.g. IL-1, IL-2, IL-3 and IFNs) are co-regulated, displaying extensive positive and negative feedback. The specific appeal of chemokines or their receptors as experimental outcomes in human biology is their ease and reproducibility of detection. In human populations – inherently challenging targets to study as environmental exposure to adjuvants, Ag composition, dose and exposure kinetics are uncontrolled – chemokine responses are frequently 5–50-fold higher than are those of cytokines. Although antigen-driven IL-5, IL-9 and IL-13 expression *in vivo* (plasma) or directly *ex vivo* (antigen-stimulated primary culture) is often low or undetectable in a significant proportion of any population under study, type 2 immune activation is visible as substantial levels of CCL17 and CCL22 production. Similarly, the three most widely used CXCR3 chemokines (CXCL9, CXCL10 and

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Cite this as: K. T. HayGlass, *Clinical & Experimental Allergy*, 2012 (42) 1546–1548.

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CXCL11) offer more frequently detected and more intense (i.e. reproducible) indicators of IFN γ production. Although no signature chemokine has yet been identified to track Th17 activity in humans, circulating CD161(+) Th17 cells characteristically express high levels of chemokine receptor CCR6 [2]. Thus, altered chemokine or receptor expression can offer excellent indicators of Ag-specific T cell activation patterns *in vivo* to complement those obtained by antigen-specific or polyclonally elicited cytokine production in culture *ex vivo* [3].

The utility of these surrogate markers of immune activation and biological status is not restricted to analysis of adaptive responses. Elevated serum IL-6 has been used extensively in cardiovascular and other literature as an indicator of inflammation or, in some areas, of the intensity of global innate activation. Interestingly, however, the primary research data frequently reveal that serum or plasma IL-6 levels in such studies are in the low pg/mL levels, at or near detection and confidence limits of the assays employed. In contrast, serum CCL2 (MCP-1), a chemokine whose up-regulation is strongly associated with acute inflammatory processes, is evident even in healthy individuals at levels about one hundred fold higher. Upon inflammation, CCL2 exhibits much stronger, more readily quantified increases, hence better opportunities to distinguish between clinically distinct populations.

Using this strategy, Bisgaard and colleagues report findings from the Copenhagen Prospective Studies on Asthma in Childhood longitudinal birth cohort. In Cord Blood Th2-related Chemokine CCL22 Levels Associate with Elevated Total-IgE during Preschool Age [1], they seek linkages within a high risk birth cohort between cord blood indicators of type 1 (CXCL10, CXCL11) and type 2 (CCL17, CCL22) activity at birth and the subsequent development of numerous immunological and clinical outcomes over the following 6 years. They found that CCL22 levels at birth were significantly associated with total serum IgE levels measured at 6, 18 months, 4 and 6 years. Conversely, the intensity of type 1 immunity, as indicated by expression levels of the CXCR3 chemokines examined, was not related to IgE levels.

Implications

The opportunity to quantitatively relate levels of systemic type 2 cytokine/chemokine production to increased IgE levels occurring later in life is important because, while we take it as a given in the 30+ years since BSF-1/IL-4 was discovered to cause IgE class switching *in vitro* [4], longitudinal evidence prospectively linking these *in vivo* outcomes in diverse human populations has in fact been scant. Seeing a clear connection between our sophisticated but sometimes reduc-

tionist experimental systems and the events that play out over time in human populations is reassuring.

Secondly, while a highly significant association at the population level ($OR > 1.5$, $P < 0.0001$) was identified between CCL22 and total IgE levels in this high risk cohort as a whole, there was great variance in the predictive utility of this measure at the level of the individual. The positive and negative predictive values, and the area under the curve that would be obtained, indicate that this is a useful hypothesis generating effort. Predicting IgE levels at 6 months, or at 6 years, for a particular child based on their cord CCL22 levels would not be feasible.

Finally and most importantly, the identified linkage between CCL22 levels at birth and subsequent development of IgE, and to a lesser extent eosinophilia, did not demonstrate a capacity to predict changes in clinically relevant outcomes such as specific IgE levels, sensitization patterns or the appearance of clinically apparent asthma, eczema or allergic rhinitis over the term examined. Although there is much evidence linking elevated total IgE levels and current allergic asthma, Følsgaard's findings are similar to those obtained in GWAS studies arguing that total IgE levels are not associated with asthma [5] and studies of polyclonally stimulated adaptive cytokine production by cord blood cells [3].

Although disappointing, these failures to strongly link differential expression of one specific variable at birth with development of asthma in later life are unlikely to be surprising to many. The complexity of immune regulation underlying responses to antigen stimulation makes it increasingly clear that identifying a single predictive biomarker at birth – or by extrapolation a single cytokine target for therapeutic intervention – is improbable. Each genetic variant, or the expression level of any given regulatory molecule, makes an incremental contribution to the ultimate clinical outcome. This constrains our capacity to use such candidate surrogate markers for predictive or diagnostic purposes.

What are the major impediments to moving forward?

One major challenge is achieving better understanding of which combination of the many continuously variable, objectively quantified immune and physiological read-outs might best predict outcomes. Many groups are currently engaged in this task. A second, arguably greater, challenge is the problem of trying to solve one equation with multiple variables. The clinical outcomes used for patient stratification are themselves a moving target. Although asthma has long been recognized as a heterogeneous disease, just how heterogeneous it truly is – hence how difficult it is to recruit a homogeneous population for mechanistic study – is increasingly obvious [6,7].

Establishing asthma definitions, or even reaching consensus on which criteria are most important to incorporate in such definitions, is challenging. Even the (apparently) simple approach of defining severe asthma retrospectively by utilizing the extremely clear cut end-point of death is problematic as in practice up to 50% of asthma fatalities occur in people with apparently mild to moderate disease [6,8]. A 3-year observational cohort study of almost 5000 individuals with severe or difficult-to-treat asthma was established to better characterize the natural history of severe or 'difficult-to-treat' asthma [9]. It incorporated a range of candidate biomarkers in a rigorous multi-centre design. Recent exacerbation history was found to be the strongest predictor of future asthma exacerbations. Thus, the best predictor of future behaviour continues to be past behaviour – not any of the extensive panel of candidate surrogate markers for which we hold out hope. Much attention now focuses on this issue [10,11].

One likely reason behind these difficulties is that a variety of endotypes are incorporated within an operationally defined phenotype, 'contaminating' otherwise homogeneous groups. An endotype is a subtype of a condition that arises from distinct pathophysiological pathways, whereas definition of an operational phenotype is arrived at via consensus derived, observable characteristics without implication of the responsible mechanisms. Thus, even carefully stratifying a popula-

tion to obtain an apparently homogeneous phenotype yields multiple endotypes, or subgroups of individuals who arrived at, for instance, severe allergic asthma via mechanisms, which exhibit immunological, physiological, pharmacological and clinical diversity [7].

Where next?

Ongoing efforts to achieve improved means of stratifying asthmatics into more precisely delineated groups will be key to enhancing our ability to tease out immune and physiological predictors. More homogeneous populations will facilitate the search for improved markers that associate with specific endotypes, reducing or eliminating the confounding effects that are an inescapable outcome of studying mixed populations. Finally, given that < 1% of human cohort studies focused on inflammatory disorders have utilized chemokine responses as readouts, opportunity seems to exist in that strategy.

Acknowledgements

I thank Anita Kozyrskyj and Malcolm Sears for critical reviews of this manuscript.

Conflict of interests: The author has no conflicts of interests to declare.

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