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Throughout 2018, the publications of the AJRCCM and associated ATS journals have continued to focus on the individual and societal impact of asthma and the challenges involved in managing this prevalent, but heterogeneous, condition. Asthma remains the most common chronic respiratory condition with ongoing significant unmet need at all levels of severity. The cardinal features of asthma, i.e. that it affects a significant proportion of all age-groups, but generates highly individual effects on health and socioeconomic factors, have hampered previous attempts to gauge its true cost at a population level. Nurmagambetov et al. approached this task by utilising data from the 2008-2013 household component of the Medical Expenditure Panel Survey, examining a total sample size of more than 200,000 persons (more than 10,000 of whom had ‘treated asthma’)[1]. Application of a two-part regression model indicated the cost of asthma in the USA in 2013 to be $81.9 billion, underlining the huge potential for improvements in asthma care to benefit individuals and populations in multiple aspects, including financially.

Models of airways disease.

An important step toward improving asthma care has been to deepen our understanding of the underlying mechanisms, with the ultimate aim of moving towards disease modification or prevention. The development of appropriate models is both challenging and complex, and many researchers are actively seeking novel approaches that can be successfully translated into positive outcomes for patients. In 2018, several varied and insightful in vivo and in vitro models have been used to examine the pathophysiology and putative treatment strategies for airway disease.

In vivo models of infection

There is evidence suggesting that worsening asthma symptoms in children are associated with M. pneumoniae[2, 3] and this bacterial spp. may also be involved in asthma development[4]. In order to investigate this, Masellli and colleagues described a novel model to investigate the relationship
between *Mycoplasma pneumoniae* infection and the development of an asthma-like phenotype in non-human primates[5]. This asthma-like model has many of the hallmarks of asthma with increased inflammatory signals and eosinophilic infiltration. Importantly, the authors showed that this outcome could also be achieved by the instillation of community-acquired respiratory distress syndrome toxin (CARDS) that is produced by *M. pneumoniae*, and has been detected in airway secretions from children with refractory asthma[6]. This study therefore highlights a putative mechanism to explain the relationship between *M. pneumoniae* and the development of asthma. The utility of non-human primate models was also explored in an *Ascaris suum*-induced allergic model of asthma[7]. Using metabolomics and proteomics, Camateros and colleagues showed that a novel TLR 7/8 ligand, S28463 (resiquimod), could attenuate airway inflammation, serum IgE and reduce airway hyperresponsiveness. The translational value of this model is yet to be explored, but appears to have advantages over conventional murine and other rodent models and has highlighted a potential therapy for allergic airway disease[8].

Although non-human primate models may be physiologically closer to humans, there remain advantages to using murine systems to unravel the complex mechanisms driving airway diseases. Mice can be genetically manipulated more easily to investigate specific mechanisms and pathways, and in much larger numbers, than would be feasible in larger animals. A good example of this approach was reported by Causton and colleagues[9][10], who specifically knocked down the expression of CARMA3, a scaffold protein, in airway epithelial cells of mice and demonstrated that allergic lung inflammation induced by *Alternaria alternate*, could be suppressed. Importantly, they translated these findings into human airway epithelial cells and suggested that CARMA3 acts by binding to inositol 1,4,5-trisphosphate receptor 3 and regulating intracellular Ca$^{2+}$. Such studies give valuable insight into the role of the epithelium in the innate immune response and how this may be modulating airway responses in asthma.
In vivo studies of obese asthma phenotypes

Obesity is a risk factor for the development of asthma and obese asthmatics appear to be more sensitive to environmental challenges such as ozone[11]. Using a murine model, Mathews and colleagues confirmed existing data that obese mice have increased airway hyperresponsiveness when exposed to ozone compared to lean mice[12]. However, their novel finding was that this response could be ameliorated by an antibody against IL-17, an effect that was not seen in the lean mice. Similar effects were also observed with an anti-gastrin releasing peptide (GRP) antibody. This exciting observation suggests a link between the pulmonary neuroendocrine cells that produce GRP and airway inflammation[13], highlighting additional mechanisms that may be driving the asthmatic phenotype in obese patients via a neuroendocrine axis, but also offering potential therapeutic possibilities. Further insights into the mechanisms of airway disease have also come from another study using ozone-exposed mice as a model. Cho and colleagues[14] examined the relationship of the gut microbiome to airway hyperresponsiveness. The team demonstrated that mice given antibiotics and exposed to ozone showed reduced airway hyperresponsiveness and reduced bronchoalveolar lavage neutrophils. The authors then performed microbiome analysis and showed that airway hyperresponsiveness was associated with two genera of bacteria, *Ruminococcus* and *Coprococcus*. This study clearly demonstrated a link between gut microflora and airway responses and went further to demonstrate that this effect may be mediated by short chain fatty acids. This study requires further validation but may suggest a gut-to-lung axis regulating airway disease[15].

Murine models can also be used to further explore the underlying immunological pathways regulating airways inflammation and have recently given insight into the role of the platelet in regulation of allergen sensitization in the airway[16]. Indeed, Amison and colleagues demonstrated, for the first time, that platelets can be found in the extravascular space associated with CD11c⁺ dendritic cells in sensitised animals. Furthermore, temporal depletion of platelets during allergen sensitization
attenuated the responses. The mechanisms underlying this remain unclear but this study clearly suggests a novel regulatory pathway of allergen sensitization in the airway[17]. The use of the allergen sensitization model has been used extensively in asthma research and Lin et al (Am J Respir Cell Mol Biol 58 745-755) showed that the asthmatic phenotype could be attenuated in β-arrestin-2 knockout mice. They hypothesised this occurred by reducing migration of CCR4 expressing Th2 cells which they confirmed ex-vivo. They also identified that this response was mediated by p38 MAP kinase and rho kinase signalling that may be amenable to pharmacological intervention[18]. Using a similar sensitization model, Lortie and colleagues [19] demonstrated the role of the sialomucin, CD34 in driving airway hyperresponsiveness. This molecule is expressed on a number of cells including mast cells and eosinophils. This study used cd34 -/- mice and demonstrated that this molecule was important in driving mast cell degranulation and mucus secretion, again highlighting a novel therapeutic target.

Airway cells

Although in vivo models of airway disease are important in understanding the pathophysiology of airway disease, the cellular and molecular mechanisms underlying this pathology are often studied in vitro. In 2018 we have increased our knowledge as to how airway smooth muscle cells respond to external stimuli including β2-agonists. Johnstone and colleagues identified the presence of PDE8 in human airway smooth muscle cells and importantly demonstrated how this enzyme regulates a specific intracellular pool of cAMP to mediate the action of β2-adrenergic receptor activity[20]. The mechanism(s) by which these cells contract and mediate airway hyperresponsiveness has also been examined recently using precision cut lung slices and Ojiaku and colleagues demonstrated that TGF-β1 may play an important role in mediating contractility via increasing basal and carbachol-induced bronchoconstriction[21] via a smad3 dependent mechanism. These data would suggest links between airway injury–repair responses and bronchial hyperreactivity in asthma [22].
The airway epithelium represents the first barrier to any inhaled pathogens or therapeutics encounter but also releases factors that may affect other cell types in the airway. To this end, James and colleagues examined bronchial airway epithelial cells from non-asthmatic children and asthmatic children, showing cells from asthmatic children produced lower levels of an activin A inhibitor, follistatin-like 3 (FSTL3)[23]. To test the relevance of this observation, cell supernatants from these asthmatic cells were used to stimulate fibroblasts and showed that the asthmatic supernatants stimulated collagen and α smooth actin production in the fibroblasts. This effect could be mimicked by supernatants taken from healthy cells in which FSTL3 was knocked down. However, the higher levels of FSTL3 could reflect an anti-inflammatory feedback response, thus further work is needed to understand the mechanism [24]. Similarly, using epithelial cells from severe asthmatics, Salter and colleagues demonstrated that supernatants from these cultures could stimulate eosinophil differentiation and used a blocking antibody to demonstrate that this may be mediated by thymic stromal lymphopoietin (TSLP)[25]. These studies clearly demonstrate that the airway epithelium generates mediators that drive and mediate changes in other structural and inflammatory cells in the lung and therefore may be the key cell type mediating the asthmatic response.

Our understanding as to how to use models of disease, both in vivo and in vitro, is now developing and allowing us to investigate very specific pathways and mechanisms underpinning airways disease. The use of non-human primates is challenging but offers great insight into aspects of disease that is difficult to study. The choice of model, whether it be in vivo or in vitro, must always have a translational context and relevance to the human disease in question. In 2018, these studies have generated a number of exciting avenues that may now lead to new therapeutic opportunities for human disease.

**Early life exposures and developmental trajectories in asthma**
The natural history of asthma remains poorly understood and one key area of interest to further our understanding are early life exposures, both in utero and early childhood, which have important effects on inflammatory pathways and the future development of asthma. Given the known adverse effects of in utero exposure to maternal tobacco smoking and the recent attention to electronic ("e-"") cigarettes as a potentially “healthier” alternative, Chen et al. used a mouse model to examine the effects of e-cigarette exposure during pregnancy on inflammatory responses of female mice and their offspring[26]. They exposed female mice to either room air, e-cigarette liquid without nicotine, or e-cigarette liquid with nicotine for 6 weeks prior to gestation, during gestation, and during lactation. Investigators noted higher levels of pro-inflammatory cytokines IL-1β and TNF-α in e-cigarette exposed mothers compared to mice only exposed to room air. In offspring mice, they also found higher levels of TNF-α but lower levels of IL-1β among both e-cigarette groups compared to the room air group. E-cigarette exposure (regardless of nicotine status) was also associated with upregulation of PDGF α, a marker of lung development that has been linked to lung fibrosis. Perhaps most notably, maternal e-cigarette exposure was associated with twice the level of global DNA methylation among the nicotine(+) group and three times the level of global DNA methylation among the nicotine(-) group. Taken together investigators found that maternal e-cigarette exposure was associated with alterations in inflammatory pathways that differed between mothers and their offspring plus epigenetic changes in the offspring, and that these changes were only partially related to nicotine.

Wright et al. analyzed data from the longitudinal Project Viva birth cohort in which maternal sugar-sweetened beverage and fructose consumption was obtained during the 1st and 2nd trimesters of pregnancy, childhood sugary beverage and overall fructose intake was ascertained at age 3, and current asthma status was obtained in middle childhood[27]. In multivariable models that adjusted for child BMI z-score, maternal sugar sweetened beverage intake was associated with middle childhood asthma (highest exposure quartile OR 1.68, 95% CI 1.07-2.65) and childhood total dietary fructose intake was significantly associated with increased odds of middle childhood asthma (highest
quartile OR 1.77, 95% CI 1.06-2.95). This study provides important motivation to continue research into intergenerational associations between maternal dietary intake and childhood asthma[28].

Several exciting studies on developmental trajectories leading to asthma development were published in 2018. In a post-hoc analysis of data from the population based, prospective Tucson Infant Immune Study cohort, investigators noted that among 394 mother-child pairs, 3rd trimester mitogen-stimulated peripheral blood monocyte (PBMC) Th1/Th2 cytokine patterns were associated with asthma development in offspring children at age 5, but only among mothers without asthma[29]. Specifically, inverse ratios of 3rd trimester PBMC IFN-γ/IL-13 and IFN-γ/IL-4 from pregnant mothers without asthma predicted childhood asthma but not early life eczema, childhood allergic rhinitis, allergen skin-test reactivity, or IgE at age 5. The relation of maternal cytokine ratios to childhood asthma was present in both aeroallergen skin-test positive and negative children and persisted in models adjusted for the child’s own 3-month cytokine ratio. In contrast, ratios of Th1 to Th2 cytokines in asthmatic mothers were not associated –positively or negatively – with asthma risk in the children of those mothers. This study highlights the importance of the complex relationship between maternal and fetal immune systems[30].

Two studies this year looked at early life growth patterns and future risk of asthma and/or lower airway obstruction. Investigators from the Generation R study reported 2 distinct early life growth-related pathways to lower airway obstruction at age 10: restricted fetal weight gain, and accelerated prenatal weight gain that is maintained in infancy[31]. While lower airway obstruction at age 10 was not associated with a physician diagnosis of asthma in this cohort, this may be related to the characteristics intrinsic to the cohort (a mostly white, European birth cohort with an unusually low prevalence [5.7%] of asthma at age 10). More importantly, the Generation R Cohort’s emphasis on early life origins of adult disease raises the question of whether this study offers insights into early opportunities to intervene on pathways leading towards the development of COPD[32]. A second study examined the role of infant weight gain patterns among low income, urban minority children in
the Boston Birth Cohort[33]. Among this population of children with high rates of poverty, environmental triggers, etc., investigators found that “extremely rapid” infant weight gain (compared with “on track” weight gain) during 3 intervals from 0-24 months were each significantly associated with increased odds of having current asthma after 6 years of age, even after adjustment for childhood BMI.(9) Premature birth, maternal asthma, maternal smoking status during pregnancy, and child’s sex did not modify the association between accelerated early life weight gain and asthma.

Other features in young children besides growth patterns offer opportunities for early identification and understanding of pathways that may lead to airway disease in middle childhood and beyond. Bonato et al. followed 74 children who had clinically indicated bronchoscopies at a mean (+ SD)age of 3.8 (+1) years to understand which early life clinical and pathologic characteristics might predict asthma at a follow-up encounter 5.5 (SD +2.6) years later[34]. In multivariable models, low birth weight, multi-trigger wheezing (defined as wheezing during and between exacerbations, versus episodic or no wheezing), and endobronchial biopsy-proven basement membrane thickening were the 3 factors that predicted the development of asthma while age of symptom onset, gender, total serum IgE, blood eosinophil count, parental smoking, and tissue or BAL inflammatory cell content did not. As described in an accompanying editorial, it appears that for the children with eventual asthma in this clinical cohort airway remodeling preceded airway inflammation, highlighting a need for better understanding of pathways and biomarkers to identify those at risk and assess response to therapy[35]. Among 6,128 7-year-olds who were followed for 46 years in the Tasmanian Longitudinal Health Study, Bui and colleagues used latent class analysis to identify childhood clinical, lung function, and environmental risk factor profiles that predicted COPD and active asthma at age 53[36]. Different combinations of frequent and even infrequent asthma symptoms (versus no asthma), history of allergic disease, and parental smoke exposure at age 7 were all associated with increased odds of COPD; having “frequent asthma” in childhood was associated with having COPD plus concurrent asthma – as well as the lowest levels of lung function - in adulthood. These childhood profiles
remained associated with COPD, asthma, and lower lung function regardless of current or prior personal smoking. Taken together these studies again highlight the need for better understanding of early life origins of adult disease with the ultimate goal of primary prevention.

**Environmental exposures and asthma**

The importance of pollution as a cause of asthma exacerbations is now well recognised and whilst the adverse effects of exposure to fine particulate matter (≤ 2.5 μm in diameter, [PM$_{2.5}$]) have been previously well described, new attention was paid in 2018 to the impacts of ambient coarse (PM$_{2.5}$ and PM$_{10}$) particle exposure among communities with a disproportionate burden of asthma morbidity. In a study that correlated Medicaid asthma health care utilization claims data from 7.8 million children in 34 states with data from PM$_{2.5}$ and PM$_{10}$ monitors in 834 and 518 zip codes respectively, Keet et al. determined that modest increases in both categories of ambient coarse particulate matter concentrations were associated with significant increases in asthma prevalence, emergency department visits, and hospitalizations[37]. This finding persisted in both rural and urban areas. Drilling down specifically into the impact of PM$_{10}$ on a rural, high altitude community in Colorado, James et al. found that elevated 3-day and 5-day averages for PM$_{10}$ levels were associated with increases in daily hospital visit counts for respiratory conditions in general and for asthma specifically[38]. Hopefully, these studies have begun to draw attention to an understudied modifiable risk factor for respiratory disease. While some sources of PM$_{10}$ exposure (i.e. dust storms) would be hard to regulate, other interventions, such as replacing rock salt with salt water brine to de-ice roads, are within reach[39].

Other neighbourhood level factors been associated with asthma, such as the extent to which the environment promotes physical activity. Investigators from Ontario, Canada rated 524 census tracts for “walkability” (domains included population density, dwelling density, access to retail and services,
and street connectivity)[40]. They found that among >326,000 children born in Toronto, the highest incidence of asthma occurred in the census tracts in the lowest walkability quintiles, and that health care use for asthma in a given year was more likely in children who lived in a low walkability neighbourhood during that year. Making community level improvements to promote walkability could have multiple beneficial effects, including reducing morbidity from asthma.

**Imaging in asthma**

Clinical asthma management lags behind other pulmonary specialties in the use of imaging modalities. Thoracic imaging offers an opportunity to objectively identify and quantify disease features in asthma. Magnetic resonance imaging (MRI) does not expose patients to ionising radiation and therefore more easily facilitates serial imaging: Another advantage in a long term and variable condition such as asthma. For patients, imaging techniques tend to be less effort-dependent compared to more traditional measurements such as pulmonary function tests. This is particularly important in the severe asthma patient group, for whom the manoeuvres involved in pulmonary function tests can be difficult to perform and can often provoke breathlessness and cough. Although we would not suggest that imaging techniques will or should replace pulmonary function tests, the information obtained via the different modalities may complement each other.

Two studies in 2018 highlighted the potential importance of both MRI and CT in better understanding asthma symptoms and pathology. The heterogeneity of asthma, even within a given individual, was highlighted by a thought-provoking study of sputum eosinophilia and MRI in severe asthma[41]. Twenty-seven subjects with severe asthma underwent rigorous phenotyping including airway inflammation characterisation based on induced sputum cell counts. Sixteen (59.3%) subjects had induced sputum eosinophil counts of ≥3%, indicating ongoing eosinophilic bronchitis (EB). Spirometry was also obtained, with bronchodilator reversibility utilised as an indicator of smooth muscle
hyperreactivity. MRI scans (¹H and hyperpolarized ³He) were performed and the ³He MRI ventilation defect percent (VDP) was calculated as an indicator of ventilation heterogeneity. Overall, the VDP improved following administration of a bronchodilator as expected, although patients with uncontrolled EB had significantly more persistent ventilation defects compared to those with sputum eosinophil counts of <3%. Although this was a cross-sectional study, it supports a hypothesis that ventilation heterogeneity may be impacted upon by a number of independent factors including EB and smooth muscle dysfunction. In time a combination of approaches, including immunological characterisation of airway inflammation together with quantification and localisation of ventilation defects using MRI, may be used to navigate decisions regarding biologic therapy and bronchial thermoplasty for individuals with severe asthma.

Ash et al. also used imaging to provide insights into asthma pathophysiology, analysing data obtained from 227 patients in the Severe Asthma Research Programme (SARP) and 20 healthy control subjects[42]. The authors quantified pulmonary vascular pruning using ratios of the blood volumes in small blood vessels obtained by computed tomography (CT). Ash et al. found that subjects with poorly controlled asthma exhibited significantly more pulmonary vascular pruning than healthy controls and increasing asthma severity was associated with a trend toward more pruning. It is currently unclear whether in the future the quantification of vascular pruning could play a role in clinical asthma management.

**Novel therapies for asthma**

As in previous years, 2018 has delivered ongoing developments in the field of biologic therapies for severe asthma. With five anti-asthma biologics currently approved by the US Food and Drug Administration (omalizumab, mepolizumab, reslizumab, benralizumab and dupilumab), and more in phase III clinical trials, for many clinicians the pivotal question is now not ‘should I offer my patient a
biologic therapy?’, rather ‘which one?’.

An even trickier challenge is encountered when deciding whether or not to switch from one biologic to another. Pragmatic answers to such questions often rely on educated inferences due to a lack of head-to-head studies. Mukherjee et al. have provided some welcome, albeit small-scale, comparative information regarding the two currently-available anti-IL5 monoclonal antibodies, mepolizumab and reslizumab[43]. Their small study comprised ten patients with severe eosinophilic asthma, who had received subcutaneous mepolizumab for at least a year. All ten subjects underwent a washout period of at least a year, two infusions of placebo at 4-weekly intervals (Q4W) and 4 intravenous doses of reslizumab Q4W. With regards to the primary outcomes (proportional reduction in sputum and blood eosinophilia), reslizumab was deemed superior to mepolizumab. However, as Papi et al. point out in their insightful editorial, it is worth noting that the mean pre-reslizumab eosinophil counts (in both sputum and blood) were higher than the pre-mepolizumab values[44], whilst post-treatment they were similar in both groups.

The prospect of administering a biologic treatment for asthma directly to the target organ (i.e. via an inhaled/ nebulized route) has, to date, not demonstrated any advantage over systemic administration. An innovative study presented by Lightwood and colleagues may change this perception[45]. Whereas recent phase III clinical trials of systemic monoclonal antibodies targeting IL-13 have not met their primary endpoints[46, 47], the authors investigated the potential of nebulized administration of CDP7766, a Fab fragment with high affinity for IL-13. They discussed the potential advantages this strategy may offer, including better penetration of the airway epithelium and reduced systemic exposure. Thirty-two cynomolgus macaques, sensitized to A. suum, received either nebulized CDP7766 or vehicle. No adverse effects were reported and when A. suum antigen was administered, CDP7766-treated macaques exhibited blunted increases in bronchoalveolar lavage (BAL) chemokine concentrations and pulmonary resistance. The highest tested dose reduced the concentration of almost all upregulated cytokines by 78% overall, and significantly attenuated the increase in peak pulmonary resistance (on day 2 challenge although not on day 1 challenge). These results are exciting,
not just because of the effects of the CDP7766 molecule (which of course remain to be replicated in humans), but because of the wider-reaching potential of inhaled/ nebulized Fab fragments in the future paradigm of biologic therapies for asthma.

Insights into the possible mechanisms of bronchial thermoplasty (BT) were provided by a study of flow patterns in computer-simulated lungs based on whole lung specimens from control subjects and those with fatal and non-fatal asthma, obtained via the Prairie Provinces Fatal Asthma Study[48]. Donovan et al. describe how the effects of BT and of methacholine challenge were mimicked, with BT resulting in redistribution of flow patterns, and reduced spatial heterogeneity, i.e. increased flow in previously under-ventilated regions and reduced flow to previously hyperinflated areas. This mechanism may explain how the attenuation of airway smooth muscle in a few central airways by BT has clinically been shown to result in improvements in exacerbation rates and quality of life scores without consistently significantly improving FEV₁[49-51].

Amidst the relentless march to identify and hone new asthma therapies, DiMango et al. provided a timely reminder of the importance of stepping down therapy in patients with well-controlled asthma; a task which clinicians and patients frequently and understandably shy away from, apprehensive of the potential for subsequent loss of disease control, especially an increased exacerbation risk[52]. The authors analysed information obtained from >400 participants of the Long-acting Beta Agonist Step Down Study (LASST) in order to identify useful indicators associated with an increased risk of treatment failure following step-down. An emergency room visit in the previous year was a significant risk factor (HR 1.53) and the risk also increased as the percentage predicted FEV₁ decreased. These data can be used to inform shared decision-making regarding treatment step-down in asthma and the identification of individuals who may benefit from closer observation during this process.
Conclusions.

There have been many advances in our understanding of the underlying pathophysiology, disease trajectory, imaging and treatment modalities for asthma in 2018. However, we still have a long way to go. The authors envisage a diagnostic and treatment paradigm, underpinned by true personalised medicine and shared decision making, which allows for disease remission and prevention of disease progression, we hope that 2019 brings us closer to this goal.
References.


