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Asthma and Allergies: From Diagnosis-Based Approach towards Personalised Treatments

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Jadwiga Olszewska(1855–1932; a Woman Pioneer in Medicine in Serbia and Bosnia and Herzegovina). „Sitting in the first row with both hands raised up" in the company of friends in Sarajevo around 1905. By courtesy of the Department of the Old Medical Book of the Central Medical Library in Warsaw. Reference Number GBL-I-1327 (103).

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Editorial
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Asthma and Allergies: From Diagnosis-Based Approach towards Personalised Treatments

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Asthma and allergic diseases are amongst the most common chronic disorders worldwide (1). The articles in this issue of the journal are describing various aspects related to this modern "epidemics", starting with a practical guidance on how to deal with patients with severe asthma (2, 3), highlighting the advances related to virus-induced asthma exacerbations (4) and protective factors and mechanisms underpinning resistance to asthma (5, 6), then moving on to a fascinating discussion on the importance of a healthy immune status and the development thereof (7). A particular emphasis has been given to the challenges facing the low- and middle-income countries (8, 9). Finally, the focus shifts from respiratory diseases to the 'third wave' of the allergy epidemic (after increases in hay fever and asthma) – that of food (specifically peanut) allergy (10). What links them all is the need to move away from the current one-size-fits all approach to management, towards personalised treatment and prevention strategies (11, 12).

Asthma and allergic diseases encompasses a range of linked conditions which are complex and multifactorial, and may be caused by several different mechanisms which cause multiple heterogeneous clinical phenotypes (12, 13). For example, while some patients have symptoms affecting a single organ (e.g. lungs), others have symptoms encompassing multiple organs (e.g. skin, upper and lower airways) (14). The pathological mechanisms which underlie this heterogeneity are largely unknown (15, 16). The current approach to management is focused on treating the diagnosis, rather than directing a strategy towards pathological mechanisms which cause symptoms in an individual patient (17). Although there is clear evidence that asthma is not a single disease (18-22), but a spectrum of disorders which are underpinned by both common and idiosyncratic mechanisms (often called asthma endotypes (23)), the management guidelines approach is as if it is a homogeneous disease entity (12). As a result, patients from several distinct disease subtypes, caused by different mechanisms, are forced into a single group for a "trial and error" treatment (11). This diagnosis-based approach to asthma management is suboptimal for 21st century medicine (24), and there is an important cautionary lesson to be learnt from history.

We now take for granted that corticosteroids are the first-line treatment for asthma. Following the early observations of potential benefits of corticosteroids in asthma which date from the early 1950s (25), the UK Medical Research Council conducted a double-blind randomized placebo-controlled trial to formally assess their efficacy and provide evidence for their use in clinical practice (26). The results were clear and unequivocal, but

from today's perspective very surprising - corticosteroid treatment was shown to have no advantage in asthma management over placebo (26). Intrigued and surprised by these findings which he could not reconcile with his clinical experience, Dr Harry Morrow Brown (1917-2013) single-handedly conducted a trial in which he tested his hypothesis that the presence of eosinophils in nasal secretions may be a marker of positive response to corticosteroids (27). He developed a rapid consulting-room method for sputum collection, and noted that "much encouragement was often needed" for patients to produce a specimen (a sentiment shared nowadays by many of those using induced sputum to guide asthma treatment). The results of his study were striking: oral prednisolone was very effective in the treatment of asthma – but only if the sputum of the patient contained eosinophils (27). In a marked contrast, amongst patients who did not have eosinophils in their sputum, prednisolone did not improve asthma control, and Morrow Brown concluded that in these patients, the use of corticosteroids may be contraindicated (27). This extraordinary study published more than 60 years ago (and in our opinion one of the most important, if not *the* most important randomized controlled trial in asthma), was the first controlled trail in which a biomarker (sputum eosinophils) was used for stratification of patients with the doctor diagnosis of asthma to predict treatment response. Morrow Brown provided a clear and unequivocal evidence that corticosteroids are effective only for a subgroup of patients with the diagnosis of asthma, i.e. that they are not a "silver bullet" appropriate for every patient. It is clear that the prescription of corticosteroids for asthma should be based on the mechanism which gives rise to a clinical manifestation (eosinophilic airway inflammation), rather than on a simple doctor diagnosis of a symptom-based phenotype (28).

Unfortunately, these astonishing results attracted very little interest and were largely overlooked (29). Sixty years later, the Individualized Therapy for Asthma in Toddlers (INFANT) trial (30) provided more evidence that fundamentally different mechanisms often underpin the same diagnosis in different paediatric patients. In pre-school children aged 12 months to 5 years with a doctor-diagnosed asthma, daily use of inhaled corticosteroids (ICS) preferentially benefitted children who were sensitised to inhalant allergen and had blood eosinophil count $\geq$300/μ (30). Unfortunately, most young children with doctor-diagnosed asthma or recurrent wheezing, particularly on the severe end of the spectrum, are prescribed ICS based on clinical history alone, without carrying any investigations (31). For example, the European Respiratory Society Task Force recommended that preschool wheezing should be managed according to the clinical phenotype, in that the Episodic viral wheeze (EVW) should be treated with as required short-acting bronchodilators, while ICS should be the first-line treatment for the multiple-trigger wheeze (MTW), but may also be considered in all patients with frequent or severe episodes of wheezing (32, 33). Although recommendations include discontinuing treatment if there is no benefit (33), this is rarely implemented in clinical practice (31). However, a clear distinction between EVW and MTW is difficult in many patients (33) and phenotypes in individual patients often changes over time (34, 35), making this phenotyping based only on anamnesis of limited value for treatment decision making. Two randomised placebo-controlled trials in infants who were mostly non-atopic did not show significant benefits of ICS for children in the first year of life with recurrent wheeze, but have indirectly suggested potential adverse outcomes for those in the ICS arm, including diminished lung function by school age (36), and a non-significant trend towards more symptoms (37). Diligence is therefore needed before ICS prescription in pre-school children, especially in non-atopic children in the first year of life, even if they have relatively severe symptoms. Full blood count (FBC) and skin tests to assess allergic sensitisation are simple and relatively cheap procedures, and although these two biomarkers clearly do not explain the complexity of pre-school wheezing, we would suggest that every preschool child who is considered for a treatment with ICS should have these two biomarkers objectively assessed before

commencing the treatment (38). If positive, ICS treatment should be commenced. However, if both are negative, ICS are unlikely to be of benefit, and alternative management strategies should be considered (including bronchodilators and/or muscarinic antagonists (39), or perhaps macrolide antibiotics (40)). However, biomarkers for targeted treatment among non-atopic pre-school wheezers remain elusive.

Severe asthma continues to pose one the greatest challenges faced by front-line clinicians (41). Andrew Bush describes a systematic approach to the management of a child with problematic severe asthma (PSA) who is not responding to the prescribed treatment (2), with a key message for those managing such patients: Do not rush into prescribing more treatments, but first ask 'What is it about this child which is making him/her non-responsive to standard therapies?'. Most severe asthma is associated with extrapulmonary comorbidities and social/environmental factors, rather than a genuinely therapy-resistant disease, and it is of critical importance to get the basics of management right before escalating the treatment (2). This includes ascertainment of co-morbidities (including obesity, exercise induced laryngeal obstruction, rhinitis etc), and environmental and lifestyle factors (including but not limited to adherence with treatment, exposure to allergens to which the child is sensitised, active and passive tobacco smoke exposure, etc.).

Understanding that one-size-fits-all approach patients with severe asthma is suboptimal, Scotney and Saglani outline a framework for an evidence-based approach for the diagnosis and management of children with PSA, who have uncontrolled asthma symptoms, despite maximal prescribed asthma treatment (3). The protocol has been developed and is continuously updated by the multidisciplinary team (MDT) at the Royal Brompton Hospital, London, and is of great value for all colleagues who carry out specialist respiratory assessment of children with troublesome asthma. The first step is confirming the diagnosis using *objective evidence*, which should include lung function tests (spirometry, bronchodilator reversibility and/or peak expiratory flow variability), assessment of airway inflammation (by FeNO measurement) and airway hyper-responsiveness (e.g. by direct methacholine or histamine challenge, or indirect airway challenge using exercise, mannitol or hypertonic saline). Assessment of adherence with ICS treatment is the crucially important next step. A multidisciplinary team (MDT) approach is essential to identify patients with initial diagnosis of PSA who have uncontrolled symptoms due to factors which can be modified, including poor treatment adherence, poor inhaler technique, exposure to environmental allergens or tobacco smoke, and/or treatable co-morbid conditions and psycho-social factors (2, 3). These patients with modifiable risk factors comprise ~80% of patients with troublesome asthma. However, there remains ~20% of children with PSA who have a genuinely severe therapy resistant asthma (STRA), and have poor symptom control despite good treatment adherence and correction of modifiable factors (3). Further investigation of children with STRA should include an assessment of systemic steroid responsiveness (e.g. by giving a single dose of intramuscular triamcinolone and measuring a change in asthma symptoms and objective markers such as lung function). This step is important for confirming the diagnosis of STRA, and guiding the choice of additional treatment with expensive biologics (3).

Asthma exacerbations (asthma attacks) are another domain of the disease which is associated with worse health-related quality of life, and a marked increases in healthcare-related expenditure for both patients and healthcare systems (42, 43). Children with frequent severe exacerbations have poorer long-term outcomes, including loss of lung function during school-age years and a diminished lung function in early adulthood (44-46). Therefore, the prediction, prevention and treatment of asthma attacks remain the key unmet needs (47, 48). However, preventing asthma attacks remains difficult (49), and large randomised controlled trial in school-age children has conclusively shown that commonly used strategy of increasing the dose of ICS at the early signs of loss of asthma control (in this case quintupling the dose)

did not prevent subsequent exacerbation (50). When discussing causation and mechanisms of asthma attacks, the focus is usually on virus infections. Kumar et al. (4) discuss the latest developments in research relating to virus-induced asthma exacerbations, including recent advances in treatment options. A substantial body of evidence suggests that deficiencies in the host innate immune response to some (but not all) viruses may predispose many asthma patients to virus-induced exacerbations. There have been several recent advances in our understanding of the mechanisms underlying virus-induced airway inflammation in asthma, including growing evidence around the interaction between viruses and bacterial infections, the role of pro-inflammatory cytokines such as IL-33, neutrophil extracellular traps (NETs) s, and eosinophils, which led to identification of novel therapeutic targets for more efficacious therapies to prevent and treat virus-induced asthma attacks (4).

While rhinovirus infections are undoubtedly important (and Kumar et al. provide an excellent overview of the role of viruses in asthma attacks) (4), they are not the only trigger of asthma attacks. We have described two peaks of acute asthma attack leading to hospital admission in Manchester, UK: one, occurring in September associated with rhinovirus infections, and a second peak in June/July, which coincided with high grass pollen level (51). The risk factors among children admitted to hospital during these two peaks of hospitalisations were fundamentally different, with most acute asthma attacks in summer occurring in children sensitized to pollen who were not using preventative treatment with ICS. We would argue that children admitted to hospital during the June/July have a different type of asthma than those who experience exacerbation in autumn, yet both have the same diagnosis and are labelled the same way. The Melbourne epidemic thunderstorm asthma which occurred on 21st November 2016 is a fascinating natural experiment which offers clues about asthma endotypes. Over a 30-hour period, there was a huge 7-fold increase in respiratory presentations to public hospitals, 476 excess asthma-related hospital admissions, and tragically 10 deaths (52). However, more than half of patients admitted to hospitals with asthma attacks had not have a prior asthma diagnosis, although most had a history of rhinitis (53). It is very likely that the thunderstorm gust front coupled with extremely high airborne ryegrass pollen concentrations created conditions for pollen grains to absorb moisture and burst into much smaller particles which can reach into the lower airways and cause allergic reaction (53). Fascinating post-hoc analysis of the open-label study of sublingual immunotherapy for ryegrass pollen sensitized patients with seasonal allergic rhinitis reported that none of the 17 participants who had completed 2-3 years of treatment and were exposed to the thunderstorm experienced an asthma attack (54). In a marked contrast, 7 of 17 (41%) control patients with allergic rhinitis who used pharmacotherapy only, had asthma attack (54). This extraordinary natural experiment offers many important learning points (53), among them the fact that most patients who were hospitalized with severe asthma attack did not have prior diagnosis of asthma, and that allergen-specific immunotherapy appeared to offer protection. Did these patients have several co-morbid conditions (seasonal asthma, seasonal allergic rhinitis and/seasonal conjunctivitis, or did they suffer from a single condition? We would argue that for most patients admitted to hospital during the thunderstorm asthma episode has a single disease with a clearly defined mechanism (ryegrass pollen IgE-mediated airways disease), that this disease has a biomarker for treatment stratification (specific IgE to ryegrass pollen), and that a mechanism-based treatment is available (specific immunotherapy for ryegrass pollen). We therefore proposed that this may be one clearly defined asthma endotype (17). This, however, is a very different disease compared to that seen in patients with recurrent attacks caused by rhinovirus, in whom very different mechanisms lead to asthma attack.

There are potential biomarkers of interest which may help differentiate virus-induced asthma attacks from non-viral exacerbations. For example, in steroid-naïve asthmatics, serum IFN-

γ-induced protein 10 (IP-10; CXCL10) levels are higher among patients with virus-induced asthma attacks compared to those with non-viral attacks, and serum IP-10 levels have been shown to have a sensitivity of 95% and specificity of 70% for virus-induced asthma (55). Ultimately, only through better understanding of the mechanisms underlying the interactions between respiratory viruses and the immune system will we be able to develop novel mechanism-based treatments to prevent and/or treat virus-induced asthma attacks (4).

That prevention is better than cure is not a new idea – it is attributed to the great Dutch philosopher and scholar Erasmus. The reduction of the burden of persistent asthma via prevention of early disease onset remains an important, but as yet elusive goal. Seminal epidemiological studies, to a large extent led by Erika von Mutius, have conclusively shown that prevalence of asthma is low amongst children in the traditional farming families. In this issue of the journal, Pechlivanis and von Mutius summarise the main finding of 27 studies which investigated the effect of farming on the risk of asthma in children (5), and discuss potential mechanisms which lead to this "acquired" asthma-resistant phenotype. The protection appears associated with prolonged exposure, not only postnatally but also antenatally, to microbial products derived from farming-related activities (5). The strongest protective effect was observed for the contact with farm animals and intake of unprocessed farm milk (56). Both of these protective exposures are associated with high and diverse microbial content (e.g. the effect of unprocessed milk consumption is abrogated by heating (57)). The epidemiological observation of prenatal protective effects of farming have been triangulated in experimental mechanistic studies in neonatal mouse models which confirmed the protective effect of exposures to a variety of microbial extracts including that from barn dust, giving us confidence that novel strategies to prevent asthma could be developed (5). Overall, the evidence to date is consistent with the proposal that microbial diversity is a hallmark of farm homes and contributes to the reduced asthma risk, and that similar farm-like microbial composition in non-farm homes also offers protection (58). In such microbe-rich environments, exposure to microorganisms from different sources can occur *via* skin, respiratory tract or gut, and this environmental microbiome shapes the host microbiome to modulate innate immune response and impact upon the risk of disease (59, 60). The current evidence on host microbiome, and its relationship with asthma in children has been summarised by Dick and Turner (6). The route of exposure may be important, but it remains unclear what is the relative contribution of the microbiome of the upper and lower airways, the gut and the skin, to immune tolerance, asthma and allergy (6). For example, some studies have suggested that nasal, but not throat microbiome is associated with reduced risk of asthma (59). A recent study from Pozega County in Croatia indirectly supports the potential important role of the gut microbiome (61). Children living in homes with drinking water supply from individual wells had a reduced risk of allergic diseases when compared to those living in homes with a public mains water supply (61). The study team capitalized on availability of data on microbial content of water during each child's first year of life to demonstrate that the risk of allergic diseases decreased with increasing bacterial content in drinking water a dose-response manner, suggesting a possible causal relationship (61). Importantly, the information gleaned from these naturally *asthma-resistant* populations and subsequent mechanistic studies offers potential pathways towards translation into the primary prevention strategies (62).

The idea of primary prevention puts emphasis to strategies for promoting health, rather than focussing on the disease. In this context, there is a common misconception that having a "strong" immune system is beneficial for health in general. However, a healthy immune status requires effective responses against pathogenic microbes and cancer cells, while at the same time it needs to tolerate self-tissues, commensal microorganisms and harmless environmental antigens, or, as a Kucuksezer et al. put it forward, the 'balanced tolerance' is essential for the survival (7). Allergic diseases,

along with autoimmune disorders and transplant rejection are examples of the consequences of the loss of immune tolerance. Therefore, understanding the mechanisms of peripheral immune tolerance is critically important, as both excessive and deficient immune tolerance lead to potentially unwanted and adverse outcomes (7). Kucuksezer et al. describe how the balance between reactivity (effector functions) and non-reactivity (tolerance) can be established for a healthy state, and argue that understanding of the relationship between innate immunity and immune tolerance, and development of biomarkers of the tolerance status are key factors for the development of novel therapeutic targets for personalized approach (7).

The focus of this special issue of the journal then moves on to the issues relevant to low- and middle-income countries (LMICs) (8, 9). Soto-Martinez et al. review the obstacles for achieving asthma control in LMICs, which include social, financial, cultural and healthcare barriers (8). There is no doubt that global asthma guidelines have played an important role in raising the awareness and improving diagnosis and management of asthma in LMICs. However, the generic guidelines are often difficult to implement in the healthcare systems overstretched by the pressure of communicable diseases. This can be in part addressed through the development and implementation of national asthma guidelines tailored to local needs, and, given the sharp increase in asthma prevalence in LMICs, this should be a public health priority in these areas (8). There are notable examples of success of such approach – for example, the implementation of the National Asthma Plan in Costa Rica, which included the provision of beclomethasone as an affordable preventive medication for all patients, has resulted in a marked decrease in hospital admissions (by 53%) and mortality (by 80%) in the country (63). Given that the main reasons for inadequate asthma control in these underserved populations include weak infrastructure of health services, low accessibility of controller medications, poor adherence, lack of education, adverse environmental exposures and social, cultural and language barriers, the emphasis and concerted effort should be focussed on improving education and access to care, including better access to effective treatments (for example, having basic preventer asthma medicines and spacers available free of charge). This, however, can be achieved only through governmental commitment (8, 63).

Lower respiratory tract illness (LRTI) caused by the respiratory syncytial virus (RSV) is a recognised risk factor for the development of asthma (64). The most hospitalisations and mortality from RSV-related diseases occur in LMICs, and Laudanno et al. review the disparities in burden and outcomes of RSV LTRI between industrialised and developing countries, and highlight the need to identify specific risk factors in different populations for a targeted RSV LRTI prevention (9). To this end, the article reviews the current state of development of several vaccines to prevent RSV infection and monoclonal antibodies to prevent severe disease, which are in the late phase of clinical trials (64). These new treatments may completely change the landscape of RSV infections in young infants globally by providing effective solutions against this important pathogen, but access to treatments needs to be secured for the areas of greatest need – namely, the low- and middle-income countries.

The final topic relates to peanut allergy, which was uncommon before the 1990s, but has risen sharply over the last two decades (65, 66), to the estimated prevalence of 2.5% among school-age children in the UK (67). Chong and Turner provide a thorough overview on the latest advances in the management and prevention of peanut allergy (10), which in recent years has firmly shifted from strict avoidance, prompt recognition of allergic reactions, and rapid initiation of adrenaline autoinjector, towards active management and prevention (68-70). The key evidence was provided by the Learning Early About Peanut (LEAP) study which demonstrated for that the early introduction of peanut into the infant diet (prior to 12 months of age) substantially reduces the risk of the subsequent development of peanut allergy (81 percent relative reduction in risk), while delayed introduction beyond 12 months increases risk (71). This

has led to the update in Guidelines for prevention of peanut allergy, which now advocate the early introduction of peanut-containing foods into the diets of infants (72). The practical information for clinicians can be found on https://www.niaid.nih.gov/sites/default/files/peanut-allergy-prevention-guidelines-clinician-summary.pdf. So, unlike in asthma where prevention is still elusive, in peanut allergy there is an emerging consensus that active preventative measures should be implemented for high-risk infants (those with severe eczema and/or egg allergy) by introducing peanut-containing foods as early as 4-6 months, but only after determining that it is safe to do so. Whether such active intervention would be appropriate for infants at moderate- or low-risk of developing peanut allergies, and/or for population-based recommendations, remains a matter of debate (70). Furthermore, the complexity of, and barriers to, effective implementation of such primary prevention interventions in resource-poor settings in LMICs cannot be underestimated (70).

For patients with established peanut allergy, approach is also gradually shifting from complete avoidance (with the provision of adrenaline auto-injectors) towards food immunotherapy as a form of *active* management. However, whether immunotherapy for food allergy is ready for the routine use in clinical practice is a subject of a considerable debate. For example, oral immunotherapy (OIT) for peanut is associated with a higher rate of allergic reactions compared to strict avoidance (73), and adverse events (which include anaphylaxis) are common and contribute to a treatment-failure/withdrawal rate of ~20% (74). A lack of data to inform safety and longer-term efficacy is a major gap in evaluating OIT for routine clinical use. A sustained unresponsiveness (i.e. an outcome of treatment in which *clinical non-reactivity to allergens persists once therapy has finished*) is a principal objective of food immunotherapy. However, the data available to date indicate that peanut OIT induces desensitisation (i.e. clinical non-reactivity *while patient is on treatment*), and consequently the majority of patients require ongoing long-term dosing (even life-long) to maintain the efficacy (74). Knowledge regarding the mechanisms of peanut OIT and the immune changes which maintain sustained unresponsiveness is limited, and addressing this knowledge gap could help identify biomarkers which could be used in treatment decisions to facilitate a personalised approach and improve safety and efficacy of food OIT (10). Therefore, before peanut immunotherapy can be considered the standard of care for peanut-allergic children, more data are needed to improve safety and longer-term outcomes (10).

The articles in this issue of AMA emphasise the need for improvements in access to care, and continuous education of physicians, patients and general public about the concept of personalised (precision or stratified) prevention and treatment strategies for common complex allergic diseases (75). We hope that this will help start the debate about these important issues tailored to local needs in Bosnia and Herzegovina.

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This Child's Asthma Appears to Be Severe: But Where Actually Is the Severe Problem?

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Abstract
The aim of this manuscript is to outline an approach to severe asthma, which is among the most challenging problems faced by paediatric pulmonologists. A logical, protocolised approach is essential. The first step is to rule out alternative diagnoses. The next step is a multidisciplinary assessment. Severe, therapy resistant asthma (STRA) is rare, and most of those referred will improve if basic management is corrected, especially adherence to treatment. However some are unable or unwilling to make necessary changes (refractory asthma plus or refractory difficult asthma). Some, especially asthma in the obese, and those thought to have STRA, progress to bronchoscopic airway phenotyping and a parenteral steroid trial to determine an individualised treatment plan. Those with persistent eosinophilc airway inflammation should be considered for omalizumab, and mepolizumab. Pauci-inflammatory asthma remains a therapeutic challenge, with a paucity of evidence; increasing steroid therapy seems neither logical nor efficacious, but options include tiotropium and azithromycin. However the most important message to the paediatrician looking after a child with apprently severe asthma is that the answer is not uncritically escalating treatment, but finding the answer to the question, what is it about this child, and his/her environment, which means there is no response to what should be easily treated airway pathology? The answer usually requires input from a skilled and experienced multi-disciplinary team, without which management is unlikely to be succesful. **Conclusion.** When managing a child with severe asthma, a detailed multi-disciplinary is essential to get the basic management right, before prescribing biologicals.

Key Words: Adherence ▪ Atopy ▪ Mepolizumab ▪ Obesity ▪ Omalizumab.

Introduction

Severe asthma is amongst the greatest challenges faced by paediatric pulmonologists – get it wrong, and you will be attending the funeral of a dead child. The aim of this manuscript is to set out a systematic approach to the management of children referred with apparently severe asthma not responding to treatment.

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What Is Severe Asthma?

In a low and middle income setting, and in pockets of some affluent societies, poor availability of basic medications is the cause of severe asthma (1). The conventional developed world definitions of severe asthma, for example the ERS/ATS Task Force (Table 1) (2), are based on levels of prescribed medication. However, it is clear that domains of risk must be incorporated, which are discussed in more detail below, the main one being risk of a severe asthma attack; a prescribed pharmacology based definition is on its own inadequate.

What Do We Know?

Four key studies should be noted (3-6):

Table 1. ERS/ATS Task Force Definition of Severe Asthma

• Asthma which is only controlled or uncontrolled on therapy with > 800 mcg/day BDP equivalent plus additional controllers (LABA, LTRA. Theophylline) or failed trials of these agents, AND any of:
1. Poor symptom control, e.g. Asthma Control Test (ACT) <20
2. ≥2 bursts of systemic corticosteroids (≥3 days each) in the previous year
3. Serious exacerbations (≥1 hospitalisation or PICU stay) in the previous year
4. Airflow limitation: FEV_1<80% predicted following SABA and LABA withhold

BDP=Beclomethasone dipropionate; $FEV_{1=F}$irst second forced expired volume; LABA=Long acting beta-2 agonist; LTRA=Leukotriene receptor antagonist; PICU= Paediatric intensive care unit; SABA:Short acting beta-2 agonist.

- The BADGER (3) study posed the question, whether the best strategy for children still symptomatic on inhaled fluticasone 100 mcg twice daily is to increase the dose of the inhaled steroid (ICS), add a long acting β-2 agonist (LABA), or adding a leukotriene receptor antagonist (LTRA). The LABA strategy was optimal, and very few children gained benefit from an increased dose of ICS. Hence this dose should be considered 'standard dose' not 'low dose', and bigger doses are 'high dose'
- In a North American inner-city study of children with uncontrolled asthma (4), aiming to answer the question as to whether exhaled nitric oxide (FeNO) in addition to standard monitoring improved asthma control, the improvement during the 2-week run-in period was so great, that there was no scope for further improvement
- Another North American study (5), which attempted to determine whether, in children symptomatic on LABA and ICS, it was better to add LTRA or azithromycin to the regime. The study ended in futility because most of the children they attempted to recruit either did not have asthma or were not taking treatment.
- A genome wide association study comparing mild with moderate-to-severe asthma in two large cohorts showed very substantial genetic overlap between the two groups in both cohorts (6), albeit with some novel severe asthma variants. I am forced to the conclusion that it is not airway pathology that makes difficult asthma difficult, but other factors (below)

The important conclusion for those managing apparently difficult asthma is that most children have an airway disease that is easy to treat, and the approach to a child with apparently therapy resistant asthma is not to prescribe more treatments, but rather ask, 'what is it about this child which is making him/her non-responsive to standard therapies?'

So, Where Usually Is the Difficulty?

Table 2 sets out the three domains which should be considered in airway diseases (7). The airway disease of asthma should be easy to treat, so social/environmental factors, and co-morbidities are likely important in severe asthma. This is borne out in the UK confidential report on asthma deaths (8); important factors were poor adherence to ICS, over-use of short acting β-2 agonist (SABA), failure to attend regular asthma reviews and repeated emergency room visits. Most deaths were in patients who were not considered to have severe asthma! Importantly, the biggest predictor of an asthma attack is a previous severe attack (9). Hence any assessment of asthma attacks must include extra-pulmonary factors, and any definition of severe asthma based solely on levels of medication must be wrong.

Assessment of the Patient with Respiratory Symptoms (a) Attributed to Asthma; and (b) Not Responding to Standard Therapy

Our approach is summarised in Figure 1. These children need a detailed, multi-disciplinary assessment (10, 11), before even considering beyond guidelines therapies, especially with biologicals.

Step 1: Is the diagnosis correct? The first question is whether the child has an airway disease at all, or is merely reporting breathlessness secondary

Table 2. Three Domains of Airway Disease to Be Considered in Severe, Therapy Resistant Asthma*

Domain of Airway Disease	Clinical Traits	Treatment (especially what is treatable)	What treatment success would look like
Pulmonary	Airway eosinophilia	Inhaled corticosteroids Omalizumab Mepolizumab	Reduction in asthma lung attacks, better baseline control
Extrapulmonary (co-morbidities, asthma plus)	Obesity	Diet Bariatric surgery	Weight loss Improved exercise performance Reduced obesity asthma
	Exercise induced laryngeal obstruction	Identification of problem, physiotherapy and sometimes psychological intervention	Better exercise tolerance, medications weaned
Environment and lifestyle	Poor adherence	Interventions to support adherence Directly observed therapy SMART regime or other simplification	More consistent use of medications, better asthma control, fewer attacks
	Exposure to allergens to which the child is sensitized	Allergen avoidance, e.g. removing pets	Better asthma control, fewer attacks
	Active and passive nicotine exposure	Referral to smoking cessation clinic	Exposure reduced, better asthma control, fewer attacks

*The problem usually does not reside in the domain of airway pathology. Examples are given, the Table is not meant to be exhaustive.

to cardiopulmonary deconditioning. In one study, around 50% of those reporting breathlessness did not have an airway disease, although many were treated for asthma (12). If the child does have a disease, the differential diagnosis is wide (Table 3), and will vary across the world; for example, airway compression from tuberculous lymph nodes is common in Cape Town, rare in London. A detailed re-evaluation, starting with a thorough history, particularly focussed on whether the child exhibits true whistling wheeze and who else has heard the wheeze, and also a physical examination, is essential. Basic investigations if not already performed should ideally include measurement of total and specific IgE and skin prick tests to determine atopic status (non-atopic school-age asthma is rare, and should prompt a complete review) (13); blood eosinophil count and FeNO to explore the possibility of ongoing eosinophilic airway inflammation; spirometry before and after SABA administration, and consideration of home peak flow measurement and a test of airway hyper-responsiveness, to determine whether there is variable airflow obstruction; should all be considered. In terms of imaging, most will have had multiple chest radiographs; we reserve CT scanning for cases where the diagnosis is in doubt.

Step 2: The child truly has asthma, so needs a multidisciplinary team assessment This will include the determination of the contributions made by co-morbidities ("asthma plus") and areas of basic management that have not been got right ("difficult asthma"), as well as assessment of the airway disease in terms of treatable traits, such as the presence or otherwise of eosinophilic inflammation and bronchodilator reversible airflow obstruction. Table 4 summarises this process of this Stage 1 assessment (10, 11). Visits to the home and at least a discussion with the school is an essential part of the evaluation.

- Asthma plus – obesity: the obese child should be evaluated particularly carefully. Firstly, breathlessness and wheezy breathing may be a sign of deconditioning (12), and not an airway disease. If there is any doubt, a cardiopulmonary exercise test with measurement of spirometry or peak flow after exercise should be performed. If there is an airway disease, it should not be assumed to be eosinophilic (14, 15) The airway may also be the target of systemic inflammation, mediated via Interleukin (IL)-6 (16). Dysanaptic airway growth (defined as a normal first second forced expired volume (FEV_1) with greater than normal forced vital

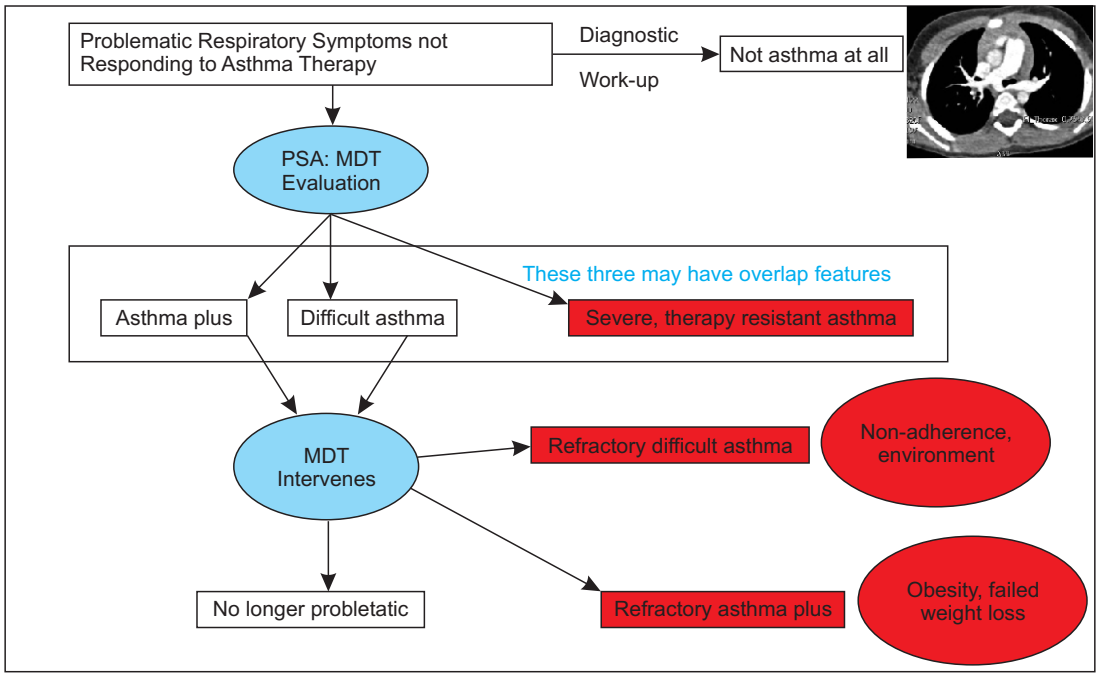

Figure 1. Approach to severe asthma. The High-resolution computed tomography (HRCT) scan shows a pulmonary artery sling, a differential diagnosis of severe asthma; MDT=Multidisciplinary team; PSA=Problematic severe asthma.

Table 3. Differential Diagnosis of Severe Asthma

Class of Diagnosis	Examples
Local (i.e. confined to the respiratory tract) immunodeficiency	Cystic fibrosis, primary ciliary dyskinesia, persistent bacterial bronchitis (cause often not found)
Systemic immunodeficiency	Any innate or adaptive immunodeficiency, including B cell and T cell dysfunction
Bronchial obstruction within the airway itself	Foreign body, carcinoid, other tumor
Obstruction arising from disease of the airway wall	Tracheobronchomalacia, complete cartilage rings, intramural tumor
Bronchial obstruction resulting from external compression	Vascular ring, pulmonary artery sling, congenital lung cyst, enlarged lymph nodes due to tumor or tuberculosis, other mediastinal masses
Direct aspiration from the pharynx	Bulbar or pseudobulbar palsy; laryngeal cleft
Aspiration by direct contamination from the oesophagus with normal pharyngeal function	H-type fistula
Aspiration secondary to gastro-oesophageal reflux	Any cause of gastroesophageal reflux, including hiatus hernia and esophageal dysmotility
Complications of 'preterm birth' or 'prematurity'	Bronchomalacia, structural secondary to intubation, vocal cord palsy secondary to surgery for patent arterial duct
Congenital or acquired heart disease	Bronchial compression from enlarged cardiac chambers or great vessels; pulmonary oedema
Interstitial lung disease	Any not presenting with neonatal respiratory failure
Dysfunctional breathing	Exercise induced laryngeal obstruction, hyperventilation syndromes

capacity (FVC) and thus a reduced FEV_1/FVC ratio) is associated with obesity and worse outcomes (17). Thus even in genuine obese asthma, ICS treatment should not be uncritically escalated, and measurement of airway inflammation at least indirectly (FeNO, induced sputum or peripheral blood eosinophil count) should be undertaken. Finally, a research area is the effects on the airway of alteration in particular in the gut microbiome in obese children (18). Treatment of obesity is of course weight loss; it may be that bariatric surgery is needed (19).

- Asthma plus – exercise induced laryngeal obstruction (EILO) (20): typically this presents as dyspnoea during rather than after exercise, which is more typical of exercise-induced bronchoconstriction. Symptoms are produced by adduction of the vocal cords, usually in inspiration. An underutilised test for EILO is a simple SMARTPHONE video recording, which typically demonstrates stridor and a tracheal tug (21). Definitive diagnosis, which is especially important if surgery is contemplated, is by laryngoscopy during exercise. A subgroup with EILO had laryngomalacia in infancy (22), or laryngeal nerve damage after ligation of a patent ductus arteriosus complicating preterm birth (23), so a detailed perinatal history should always be obtained.
- Asthma plus – rhinosinusitis: the "united airway" concept is controversial; clearly upper airway disease should be treated on its own merits, and many studies suggest this also benefits lower airway disease (24).
- Asthma plus – obstructive sleep apnoea: in our series, unless the child is also obese, this is not a feature of severe asthma.
- Asthma plus – food allergy? There is a clear association between food allergy and severe asthma (25), but whether there is causality is unclear. We do not perform blind dietary manipulations, and only diagnose food allergy with objective testing.
- Asthma plus – gastro-oesophageal reflux (GOR)? GOR is frequently detected, but there is ample evidence that treatment, whether or not there are suggestive symptoms, does not improve asthma control (26).
- Difficult asthma – adherence: this is perhaps the biggest challenge faced by Paediatric Pulmonologists. It is a waste of time to ask families if they are adherent, or ask them to fill in adherence questionnaires; the answer always is that the child takes all the treatment all the time. Sometimes this is a genuinely held be-

Table 4. Multi-Disciplinary Assessment of Severe Asthma

Issue to be Addressed	Tests Performed
Symptom pattern	Asthma control test, prednisolone bursts, unscheduled visits
Psychosocial factors	Questionnaires relating to treatment burden, anxiety and depression, quality of life
Lung function	Spirometry before and after bronchodilator
Allergic sensitization	Skin prick tests, specific IgE
• Aeroallergens	Grass and tree pollen, house dust mite, cockroach, cat and dog, and any others suggested by the clinical history
• Food allergens	Peanut, milk, egg and any others suggested by the clinical history
Fungal sensitization	See Table 6
Airway inflammation	FeNO Induced sputum cytospin if FEV_1 is >70% predicted
Nicotine exposure (tobacco or vaping)	Urine or salivary cotinine
Medication adherence	Prescription uptake Serum prednisolone and theophylline levels if prescribed; serum inhaled corticosteroid levels if available (usually only in a research context)

FeNO=Fractional expired nitric oxide; FEV_1=First second forced expired volume; IgE=immunoglobulin E.

lief by parents who do not realise they are not adequately supervising medication inhalation. There is a hierarchy of data and tests which lead to more objective understanding. The simplest is to access prescription records (27); of course, collecting a prescription is not the same as medications being properly used, but failure to collect definitively means none taken. A home visit is often also informative – oftentimes medications are found still in their original wrappings, or our out of date, or packed away at the back of a cupboard; none of which inspires confidence that medication is actually being used (27). We increasingly use electronic monitoring using Smartinhalers™. Four patterns of use have been described (Table 5) (28), each prompting a different course of action. These inhalers only monitor activation, not inhalation, and, compared with those with a microphone to detect inhalation, overestimate adherence (29). Finally and probably the gold standard, directly observed therapy (DOTS) by video link using smartphones and Bluetooth technology (30). In one study, this technique was acceptable to the patients, but, despite multiple previous attempts at teaching, it took five weeks before the children were using their inhaler devices correctly at home. In those few children prescribed prednisolone or theophyllines, measurement of blood levels may be informative; serum cortisol should also be suppressed in those on chronic prednisolone therapy, and an elevated level suggests non-adherence. Measurement of serum levels of ICS is currently only a research technique. Other medication issues include failure of parental supervision (above), and, particularly by teenagers, using metered dose inhalers without a spacer (27).

Difficult asthma – adverse environmental factors: these include persistent active and passive exposure to cigarettes and vaping devices, detected by persistently elevated urinary or salivary cotinine; referral to a smoking cessation clinic should be offered. Another factor is persistent exposure to allergens to which the child is sensitised, usually furry pets (27). Frequent comments are that the child is no worse when near the pet cat, and was no better when the cat was sent away for two weeks. However, low dose allergen exposure can drive subacute type 2 inflammation (31), synergises with viral infection to cause acute attacks (32), and it takes at least a year for cat allergen levels to drop to low levels after removal of the animal. Another issue is mould exposure, frequently accompanied by a request for a letter to support rehousing. However, environmental fungi may cause severe asthma with fungal sensitization (Table 6), which is probably IL-33 mediated and causes more severe inflammation (33).

- Difficult asthma - Psychosocial issues: these are very common in severe asthma, and the relationship between the two is complex. Rather than trying to decide which came first, it is better to address psychosocial issues in parallel with trying to treat asthma (27). It is virtually impossible to manage severe asthma without skilled psychological support.
- Difficult asthma - Symptom perception: which may involve exaggeration or downplaying of symptoms. Exaggeration may be for financial benefits (in the UK, having a sick child allows the claiming of cash benefits), or for more serious underlying reasons, for example panic attacks after the trauma of being ventilated for asthma, or hyperventilation to escape going to school or from an abusive situation at home. Some children with asthma fail to appreciate that their airways are progressively obstructing; this situation is dangerous, difficult to address, and may rarely underlie asthma deaths.
- Difficult asthma - Asthma education: sometimes ignorance of the basics of the disease may contribute to the problem, and checking basic knowledge is an important part of the assessment (27).

Step 3: After the assessment, a multi-disciplinary review followed by an intervention All the data above are collated and discussed. In the minority, no potentially reversible factors are detected, and the child progresses to invasive phenotyping and beyond guidelines therapy (true Severe, Therapy

Table 5. Patterns of Non-adherence and Their Management

Symptom/Test results	Interpretation	Actions
FEV_1 and FeNO abnormal at start of monitoring, adherent at least in activating the inhaler during monitoring, FEV_1 and FeNO normalise and symptoms improve	Previously non-adherent, and became adherent during monitoring	Intervention to support adherence, with regular re-assessment and feedback
FEV_1 and FeNO abnormal at start of monitoring, adherent during monitoring, tests remain abnormal, still symptomatic	Severe, therapy resistant asthma	Invasive airway phenotyping
	Could also be activating inhaler but not inhaling	
FEV_1 and FeNO abnormal at start of monitoring, non-adherent during monitoring, tests remain abnormal, still symptomatic	Either refractory severe asthma, or severe therapy resistant asthma who stopped medications because they do not work	Ascertain response to DOT; if no response, invasive airway phenotyping. If responds, refractory difficult asthma, consider simplifying regimes or biologicals
Poorly adherent on monitoring, but actually remains well with normal FEV_1 and FeNO	Previously over-treated	Wean asthma treatment but continue to follow up carefully

DOT=Directly observed therapy; FeNO=Fractional expired nitric oxide; FEV_1=First second forced expired volume.

Resistant Asthma, STRA, discussed in more detail below). In most, however, assignment is to difficult asthma or asthma plus, acknowledging they may overlap, and a plan is made to try to improve control and reduce attacks. These may include:

- Difficult asthma – interventions to support adherence including DOTS at school, and simplification of the regime, for example using Symbicort™ as a single preventer and reliever inhaler, or once daily Relvar™ (fluticasone furoate/vilanterol); environmental interventions such as house dust mite impermeable bed covers, removal of pets, and addressing smoking and vaping
- Difficult asthma – an admission for evaluation to hospital (34). In particular if adherence is thought to be an issue, or symptoms are overcalled, direct observation over a period time may be illuminating. Improved spirometry and a fall to normal in FeNO during hospital DOTS is strongly suggestive of previous poor adherence. SABAs are only permitted after evaluation by a paediatrician, and very often, the child is well and active without asking for them, suggestive of previous over-calling of symptoms. We discover safeguarding issues in adherence and overcalling symptoms in around 10% of children referred to our difficult asthma service
- Asthma plus – co-morbidities are tackled, particularly the aid of a specialist physiotherapist to tackle EILO, weight reduction under the supervision of a dietician, and consideration of a specialist referral to tackle upper airways disease

Step 4: Has the problem been solved? The above approaches result in many difficult asthma

Table 6. Definition of Severe Asthma with Fungal Sensitization (SAFA)

Adult Criteria	Proposed Paediatric Criteria*
Treatment with 500 µg fluticasone/day or equivalent, or continuous oral corticosteroids (less useful in the age of biologicals, since far fewer adults are prescribed oral corticosteroids on a long term basis, or four prednisolone bursts in the previous 12 months or 12 in the previous 24 months, and all of	Meets criteria for problematic severe asthma
1. IgE <1000 (exclude ABPA)	No IgE exclusion
2. Negative IgG precipitins to Aspergillus fumigatus	No IgG exclusion
3. Sensitization (SPT, sIgE) to at least one of Aspergillus fumigatus, Alternaria alternata, Cladosporium herbarum, Penicillium chrysogenum, Candida albicans, Trichophyton mentagrophytes and Botrytis cinerea	As adult criteria

*There is no agreed definition in children, but given the rarity of allergic bronchopulmonary aspergillosis (ABPA) in children with asthma, we eliminate the total IgE and IgG criteria, from the diagnostic criteria.

and asthma plus patients improving dramatically, with reduction in prescribed medications, improved asthma control and reduction in attacks, and better physiological parameters (spirometry, FeNO). These children need support to maintain their improvement. However, despite the best efforts of the team identifying problems and trying to support change, some families and children fail to engage, resulting in ongoing poor control and asthma attacks, i.e., are considered to have refractory disease (35).

- Refractory difficult asthma: the commonest reason is failure to support adherence; DOTS only works if the child goes to school regularly, is prepared to go to the school nurse's room for treatment, and the nurse is prepared to check obsessionally that the inhaler is in date and not empty, and actually closely watches the child taking the medication. Other causes are inability or unwillingness to address environmental factors, and intractable psychosocial issues
- Refractory asthma plus: usually the issue is asthma and obesity with failed weight loss; referral for bariatric surgery should be considered, but in the meantime, asthma must be addressed.

Previously we argued (incorrectly, with retrospect) that only STRA children should be eligible for biologics (36). Given that most asthma deaths are not in STRA, we now believe that children with refractory difficult asthma should also undergo invasive airway phenotyping to develop an individualised treatment plan.

Stage 2 and 3 assessment: What is the airway pathology? The aim is to determine the answers to four question:

- Is the asthma steroid responsive, or is a non-steroid based approach needed?
- Is there evidence of airway inflammation, and if so, what is its nature? For example, there seems little point in giving anti-Type 2 (TH2) agents such as mepolizumab if there is no airway eosinophilia, or increasing the dose of steroids if the airway is pauci-inflammatory?
- Is there discordance between symptoms and inflammation (37)? Either multiple symptoms with no inflammation, or eosinophilic inflammation without symptoms, but which confers a high risk of a future asthma attack?
- Is there persistent airflow limitation (PAL), in which case escalating treatment to try to restore normal lung function will only succeed in exposing the child to the risk of side-effects?

The child attends hospital for stage 2, and is assessed with spirometry with acute response to SABA (bronchodilator reversibility, BDR), FeNO, and induced sputum cytospin. The child next undergoes a fibreoptic bronchoscopy, bronchoalveolar lavage and endobronchial biopsy under general anaesthesia, during which time a single intramuscular dose of the glucocorticoid triamcinolone (40 mg if weight <40 kg, 80 mg all others) (38). The final visit, for stage 3, takes place 4 weeks later, during which all the non-invasive tests above are repeated, and the response to triamcinolone determined. We have shown that the adult definition of steroid responsiveness, namely a 15% or greater predicted increase in morning FEV_1 in patients with BDR of 12% or greater from baseline and an abnormal FEV_1 (<80% of predicted value) before a systemic steroid trial (39, 40), cannot be used in around half of children with STRA, because they have normal spirometry (38). We use a multidomain approach (Table 7), assessing symptoms, spirometry and airway inflammation (41), which is now increasingly also being adopted in adult medicine (42).

What treatments could be considered? For many children, there are no easy therapeutic answers.

- **Is the child eligible for omalizumab?** There is most paediatric evidence with the anti-IgE monoclonal omalizumab, which inhibits the binding of IgE to the mast cell and basophil high-affinity IgE receptor (FcεRI), thus limiting the release of those mediators driving the allergic response. There is also evidence that omalizumab may have anti-viral properties. To be eligible (43), serum IgE must be >30 and <1500 international units; and the child must unequivocally be shown to be sensitised to aeroallergens, especially if IgE is near the lower limit

Table 7. Domains of Steroid Responsiveness*

Modality	Response
Symptoms	ACT rises to >19/25 or 50% increase
Spirometry	FEV_1 rises to >80% predicted or 15% increase
Inflammatory: FeNO	Falls to <24 ppb
Inflammatory: Sputum eosinophils	Falls to <2.5%

*Note that the figures we have adopted are not evidence-based. It is accepted that FeNO and sputum eosinophils are not concordant (77) but it is often difficult to obtain paired sputum samples in this population (78); ACT=Asthma control test; FeNO=Fractional exhaled nitric oxide; FEV_1=First second forced expired volume; ppb=Parts per billion.

of eligibility (although there is evidence that those without sensitisation respond equally well (44)); dosage and dosing frequency (2 vs. 4 weekly) depends on body weight and level of IgE. Unfortunately, many STRA children have an IgE above this range, making them ineligible. Although IgE determines eligibility, it is in fact a poor biomarker of response. In an adult study, a high FeNO, blood eosinophil count and serum periostin (this last is not useful in children because it is released from growing bone) were predictive of a good response (45), and in a small paediatric study (46), a fall in FeNO after triamcinolone. In our hands, asthma attacks respond better than impaired day to day control. Our practice is to assess response every 16 weeks, including asthma control test (ACT), history of attacks, FeNO, induced sputum and spirometry with BDR.

- **Is the child eligible for an anti-TH2 monoclonal?** At the moment, the only one licensed in children in the UK is the anti-IL5 mepolizumab. From the available studies, to be eligible, the child must have a peripheral blood eosinophil count of at least 150/μλ, and preferably >300/μλ. As with omalizumab, it is those prone to asthma attacks which respond best (47). It is worrying, however, that evidence for efficacy and safety has been uncritically extrapolated from adults; even in those studies recruiting young people over age 12, the vast majority recruited have in fact been adults. Firstly, there is evidence from two groups that airway eosinophilia in severe paediatric asthma may not be TH2 mediated (38, 48); of course, the question is 'does it work?' not 'should it work?', but we desperately need trials in children. Secondly, the developmental biology of the eosinophil is not well understood, but at least from animal data, there is evidence that this cell has important physiological functions (49-53). For example, adipose tissue eosinophils participate in beige fat thermogenesis and glucose homeostasis through regulation of alternatively activated macrophages (49, 50); bone marrow eosinophils are required for adjuvant-induced B-cell priming and maintenance of memory plasma cells (51, 52); and resident intestinal eosinophils are distinct phenotype and constitutively express antigen-presenting cell markers (53). So it is essential that the paediatric respiratory community unite to obtain paediatric data on safety and efficacy for mepolizumab and the other upcoming anti-TH2 monoclonals (54).
- **What do we do about pauci-inflammatory asthma?** This is one of the least-understood groups. An adult proof of concept study demonstrated that this group was not steroid-responsive (55). Evidence is scant, but these children may be considered for Tiotropium (56), or given azithromycin (57) long term. Perhaps the most important is not to over-treat with steroids if there is no eosinophilic airway inflammation.

Following up: What next? The initial assessment is of course only the start of the process. Detailed follow up is essential, to determine progress and detect any side-effects of treatment. Regression of good adherence is notoriously common, especially when the child is feeling well. There is a good case to be made for a detailed annual assessment of these children, including assessment

of linear growth, assessment of lung function trajectories (58), reassessment of exposures (including to nicotine) and sensitization, and the performance of a short synacthen test to assess adrenal function. Of note, sputum cellular inflammatory phenotypes are much less stable than in adults (59), so an annual induced sputum and FeNO should be considered. Asthma attack risk should also be determined (60), and the knowledge that the growing child has of asthma and treatment should be checked.

A (Literally) Deadly Issue: The Asthma Attack Prone Child

The word exacerbation implies a mild inconvenience that is readily reversible. In fact, what we prefer to call asthma lung attacks (61, 62) may be fatal, are a warning sign that further attacks are imminent unless something is done (9), and are associated with an impaired lung growth trajectory (63, 64). Asthma lung attacks should be a 'never event' like cutting off the wrong leg in the operating room (65).

- Firstly, determine the basis on which the asthma lung attack was diagnosed; what objective measurements were made? We have often seen that a history of so-called severe asthma who were over-treated in the emergency room; one child was given intravenous salbutamol for 'asthma' despite an oxygen saturation in air of 100%! The child was hyperventilating.
- A true asthma attack needs to be treated effectively. There is no place for a standard 3- or 5-day course of prednisolone, with no review to ensure the child has responded. The child must be reviewed to ensure that recovery is complete.
- An asthma attack must not be treated as an isolated event, like lobar pneumonia (66); it should be seen as a serious occurrence on the asthma journey, and taken really seriously
- The asthma plan should be reviewed; was it followed, should it be changed?
- Medication issues: can the child use the medication delivery device? How many ICS canisters have been accessed (67)? How many SABA canisters have been dispensed (68)? Do the child and family understand the dangers of non-adherence? Should the child have SABAs removed and substituted with Symbicort ™ (69-71), or prescribed a SMART regime (72), so the combination of underuse of ICS and overuse of SABA is taken out of the equation? Should a biological (omalizumab, mepolizumab) be prescribed to reduce future lung attack risk?
- Are there environmental issues? The combination of sensitization to allergens, allergen exposure and respiratory viral infection is strongly predictive of an asthma attack (32), of which only allergen exposure can be modulated. A proof of concept, randomised double blind study demonstrated that, in children sensitised to house dust mite and who had been admitted to hospital had improved asthma outcomes if they were randomised to mite impermeable bedding covers (73). It may be that the intervention was too focused; other studies have shown the efficacy of a more multifaceted intervention (74, 75).
- The evidence relating Vitamin D deficiency to asthma attacks has a sound experimental basis but clinical evidence is less secure (76). However, it is not unreasonable to measure Vitamin D levels and prescribe supplementation if the levels are suboptimal.
- Future work should determine if a single dose of mepolizumab as part of the management of the acute attack will reduce the risk of future attacks.

Summary and Conclusions

Severe asthma is challenging, and requires a focused, protocolised approach to management. Most severe asthma relates to extrapulmonary comorbidities and social/environmental factors rather than difficult airway pathology. A systematic multi-disciplinary assessment must be used which will often resolve the problem, or place the child into one of the three categories of STRA, refractory difficult asthma, and difficult asthma

plus. Many in these categories will require bronchoscopic airway phenotyping and assessment for biologicals such as omalizumab and mepolizumab. Asthma lung attacks are a sentinel, never-event, which should prompt a detailed response to prevent recurrence. However, the over-arching final message is that almost all children with asthma can have their disease controlled well by low dose ICS if taken regularly and correctly, and adverse environmental factors are dealt with. There is no substitute for getting the basics right!

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Diagnosis and Management of Problematic Severe Asthma

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Abstract
This review will outline an evidence-based approach for diagnosing and managing children with problematic severe asthma (PSA). Children with PSA have uncontrolled asthma symptoms, despite maximal prescribed asthma treatment. These children have high morbidity and mortality and should be referred for specialist respiratory assessment and management. The first step in the assessment of a child with PSA is confirming the diagnosis of asthma using objective evidence. Following this, an assessment of inhaled corticosteroid adherence and a multi-disciplinary team approach is essential for separating difficult asthma (DA) from severe therapy resistant asthma (STRA). The majority of children have DA which entails uncontrolled asthma symptoms due to underlying modifiable factors including poor treatment adherence, poor inhaler technique, exposure to environmental allergens, co-morbid conditions and psycho-social factors. Approximately 20% of children with PSA have STRA, and have persistent asthma symptoms despite good treatment adherence and correction of modifiable factors. Children with STRA typically have multiple and severe aeroallergen sensitization, eosinophilic airway inflammation and high fraction exhaled nitric oxide (FeNO). Further investigation of children with STRA includes an assessment of systemic steroid responsiveness, this is important for confirming the diagnosis of STRA and guiding the choice of additional treatment. Biologics are an add on (immune targeted) therapy for STRA. The current biologics used in children target the T2 helper (Th2) pathway mediating eosinophilic, allergic asthma. **Conclusion.** Future clinical trials of biologics in children will be essential to help identify childhood specific biomarkers and to decide which biologic is best for which individual child.

Key Words: Paediatric ▪ Asthma.

Introduction

One in ten children in Europe have asthma (1). Most children with asthma have mild to moderate disease and can achieve good symptom control with low dose inhaled corticosteroids (ICS). However, approximately 2-5% of asthmatic children have problematic severe asthma (PSA) (2, 3). These children have uncontrolled symptoms despite being prescribed maximal standard pharmacological treatment, equivalent to step 4/5 of Global Initiative for Asthma (GINA) management guidelines (4). Children with PSA have an increased mortality, morbidity and despite their small number account for 50% of asthma related healthcare costs (5, 6). The UK National Review of Asthma Deaths observed that 17% of the asthma deaths were in patients with severe asthma, that by current international asthma management guidance, should have been under specialist respiratory care (7). For these reasons it is essential that these children are managed in a specialist setting by a multi-disciplinary team experienced in diagnosing and managing children with PSA. Current international guidance outlines when a child with problematic asthma should be referred to a respiratory specialist (4, 8).

This review will discuss the evidence based PSA pathway followed by Royal Brompton Hospital, London (9) for diagnosing and managing school aged children (6-16 years old) with PSA, with relevant reference to medical literature.

Problematic Severe Asthma

The umbrella term PSA describes all children with persistent asthma symptoms despite maximal standard pharmacological therapy (10). Unfortunately, multiple different terminologies have been used to describe children with severe asthma, which has made it difficult to compare disease outcomes in paediatric research studies. In order to unify terminology, the term PSA was proposed by the PSA in Childhood Initiative Group, a Global Allergy and Asthma European Network (GA2LEN) Task Force (10, 11) and has been adapted by the Royal Brompton Paediatric Severe Asthma diagnosis and management pathway.

Children with PSA have the following two features

i) poor symptom control defined as one or more of:
   - Chronic symptoms (most days for >3 months) or Childhood Asthma Control Test (C-ACT) score or Asthma Control Test (ACT) score <20 (12, 13)
   - Persistent Airflow obstruction (FEV_1 post bronchodilator <80%)
   - Recurrent severe asthma exacerbations (either ≥2 hospital admissions per year or ≥3 courses of high dose oral steroids for at least 3 days per year)
   - One intensive care admission requiring mechanical ventilation
ii) prescribed high-dose inhaled corticosteroids (equivalent to >800 μg/day of budesonide or fluticasone >500 μg /day) plus a long acting β2 agonist plus montelukast (or previous failed trial) or previous trial of other add on therapy such as theophylline OR require maintenance low dose oral corticosteroids. (As per GINA Asthma Step 4/5 Treatment and ERS/ATS Severe Asthma Guidance) (4, 8).

Assessment of the Child Referred with PSA

Confirming Asthma Diagnosis

The first step in the assessment of a child with PSA is to confirm the diagnosis of asthma (8). Approximately 50% of children with PSA have an existing co-morbidity, associated diagnosis, or have been wrongly diagnosed as having asthma (14). National Institute of Clinical Excellence (NICE) Asthma guidelines advise the use of symptom history and objective tests to diagnose asthma (15). It is essential to not simply rely on the history to confirm the diagnosis, but also to use objective tests for diagnosing asthma, as outlined below.

Lung Function Tests

- Spirometry- all children aged 5 and over should have spirometry. An FEV_1/FVC ratio <70% is positive for obstructive airways disease.
- Bronchodilator reversibility (BDR)- if obstructive spirometry (FEV_1/FVC ratio <70%) then BDR testing is needed to determine if it is fixed or reversible obstructive airways disease. A BDR test result is positive for reversibility if increase in FEV_1 ≥12%.
- Peak expiratory flow variability- A 2 to 4 week period of PEFR monitoring is advised if the diagnosis is uncertain after spirometry, BDR and FeNO (see below). PEFR monitoring should be done if a. normal spirometry and b. obstructive spirometry (FEV_1/FVC Ratio <70%) with negative BDR test (not reversible obstructive spirometry) and raised FeNO ≥35ppb. Spontaneous variability in PEFR ≥20% is positive for reversible obstructive airways disease.

Airway Inflammation Tests

- Fractional exhaled nitric oxide (FeNO)- FeNO testing, is an approximate measure of eosinophilic airway inflammation (16). FeNO measurement should be considered if either normal spirometry or obstructive spirometry and negative BDR testing. A FeNO result of ≥ 35ppb is supportive for airway inflammation in children.

Airway Hyper-responsiveness Tests

- Airway Challenge- Airway hyper-responsiveness can be demonstrated by direct methacholine or histamine challenge, or indirect airway challenge using exercise, mannitol or hypertonic saline. The National Institute for Health

and Care Excellence (NICE) guidance does not recommend airway hyper-responsiveness testing in children. However, in specialist respiratory centres if diagnostic uncertainty remains after lung function testing and airway inflammation testing, it is an important additional test to consider.

Initial Investigations

Tests for Atopic Status

Asthma of all severity in children is typically allergic and hence it is important to question a diagnosis of asthma if there is no objective evidence of atopic sensitisation, particularly if the child has a chronic wet cough. Alternative diagnoses that may present as wrongly labelled or misdiagnosed severe asthma, are listed in Table 1. An assessment of the child's atopic status using patient history and objective tests is also important for identifying asthma triggers in children with asthma. Objective atopic testing may be undertaken by measurement of serum total IgE and serum specific IgE tests or skin prick testing to common allergens: food allergens (peanut, milk, egg), common aeroallergens (cat, dog, grass, tree, house dust mite) and moulds *(Alternaria alternata, Penicillium notatum and Cladosporium herbarum)*.

A small proportion of children (<15%) have non-atopic severe asthma (14, 17). Risk factors associated with non-allergic asthma include family history of asthma, eczema or rhinitis; lower respiratory tract infections in childhood, damp or mould in home environment, obesity and parental smoking (18). Despite, differences in atopic status, both allergic and non-allergic asthma in adults has been shown to have similar bronchial mucosal changes and immunocellular changes (19). Investigations to support asthma diagnosis and establish airway inflammatory phenotype are essential in non-atopic children to enable targeted treatment.

Other Tests

Additional tests that should be considered as part of an initial severe asthma assessment include chest x-ray (CXR), sweat test, vitamin D level and urinary cotinine. A CXR in a child with severe asthma may show non-specific radiological findings such as hyperinflation and peri-bronchial wall thickening. However, a CXR is most useful for investigating alternative diagnoses. A high resolution CT (HRCT) should be considered if there is an atypical asthma presentation i.e. abnormal carbon monoxide transfer factor, excessive mucus production, rapid decline in lung function, non-atopic (8). A sweat test should also be considered to exclude cystic fibrosis in those without objective evidence of an asthma diagnosis.

Approximately 30% of asthmatic children have vitamin D deficiency (serum 25 (OH)D <75nmol/L) (20). Vitamin D has been proposed to have immunomodulatory effects, including antiviral and anti-inflammatory effects (21). A Cochrane Systematic Review of vitamin D in children with asthma, identified 7 randomised controlled trials including a total of 435 children. Only one trial that included 22 children measured the rate of asthma exacerbations. Overall the systematic review reported vitamin D in adults and children reduced the rate of exacerbations requiring steroids (rate ratio 0.64, 95% CI 0.46 to 0.90). However, this review also included pre-school children (22) and mainly adults. In adult asthmatics, there is convincing evidence that vitamin D supplementation can prevent asthma exacerbations (23). However, the evidence base for recommendation of vitamin D supplementation to prevent asthma exacerbations in children is limited due to small study sample sizes (24).

Passive smoke exposure in asthmatic children can trigger asthma exacerbations (25). Asthmatic children with passive smoke exposure have more frequent emergency healthcare attendances and are twice as likely to have an asthma exacerbation requiring a hospital admission (25). Hospital admission data analysis has shown that banning smoking in public places in Scotland resulted in an 18% reduction in hospital admissions (95% CI 14.7 to 21.8; P<0.001) for asthma exacerbations in children (26). Urinary or salivary cotinine level is an objective marker of passive smoke exposure

and can be used to motivate carers to stop smoking and assess whether smoking has ceased in a household.

Table 1. Differential Diagnoses to Consider when Assessing Children with Problematic Severe Asthma

Dysfunctional breathing
Tracheo-bronchomalacia
Foreign body
External airway compression
Aspiration
Bronchiectasis
Obliterative bronchiolitis
Hypersensitivity Pneumonitis
Cystic Fibrosis
Primary Ciliary Dyskinesia

Multidisciplinary Assessment

Once a diagnosis of asthma has been confirmed, further management and assessments must be undertaken using a multidisciplinary team approach. The Royal Brompton multidisciplinary paediatric asthma team is composed of specialist asthma physiotherapists, dieticians, physiologists, clinical psychologists, specialist asthma nurses, safeguarding nurses, social workers and medical doctors. A multidisciplinary team approach is also essential to determine if the child has Difficult Asthma (DA) or Severe Therapy Resistant Asthma (STRA). This guides the direction of further management towards addressing modifiable factors or the need for further investigations to determine choice of add on treatments.

Definitions

Difficult Asthma

Children with DA have poor asthma symptom control due to underlying modifiable factors. Over 50% of children with PSA have difficult to treat asthma and symptoms improve after identifying and addressing modifiable factors (27). These modifiable factors include poor treatment adherence, poor inhaler technique, exposure to environmental allergens, co-morbid conditions and psycho-social factors (27).

Severe Therapy Resistant Asthma

Children with STRA have ongoing asthma symptoms despite good adherence to high dose inhaled corticosteroids and correction of modifiable factors. Children with STRA are immunophenotypically different to children with DA (28), STRA children are resistant to high-dose inhaled corticosteroids and have persistent eosinophilic airway inflammation, high FeNO and airway remodeling (14). The majority of children (85%) with STRA will be positive for one or more allergen have very severe and multiple allergen sensitization, and typically have worse disease severity with co-existing food allergies (29).

Assessments to Exclude Difficult Asthma

Basic Inhaler Technique and Objective Assessments of Adherence to ICS

Non-adherence to inhaled corticosteroids is strongly associated with poor asthma control, increased morbidity and mortality (7). Therefore, a thorough assessment of adherence to treatment is vital, to enable interventions to improve adherence, as well as distinguish STRA from DA. This includes assessing inhaler technique and reviewing suitability of spacer device used. Approximately 40% of children have poor inhaler technique and 15% use the wrong device (27). Incorrect administration of medication is common but is easily rectifiable with expert guidance.

Good adherence is defined as at least 80% administration of prescribed inhaled corticosteroid doses (30), moderate adherence 60-80% and poor adherence <60% administration of prescribed doses (31). Adherence can be measured by an assessment of prescription uptake, which is carried out by contacting the child's local pharmacy or primary care physician to check prescription records. Prescription uptake in half of asthmatic

children referred for specialist input is suboptimal (<80%) (27). A prescription uptake assessment may be sufficient to assess adherence, since poor prescription refill and uptake confirms poor adherence. However, good uptake does not equate to good adherence as there is little relationship between prescription uptake and actual administration of medication. Therefore, adherence is better assessed objectively by using electronic monitoring devices which attach to corticosteroid inhalers. These devices record timing and number of actuations (32). Newer devices are also able to record inhalation of the drug.

A prospective cohort study in asthmatic children attending a specialist paediatric severe asthma clinic used electronic monitoring devices (EMD) to monitor adherence to prescribed inhaled corticosteroid treatment (32). Ninety-three children had EMD monitoring for a median number of 92 days (range 56-200 days) (32). The median adherence to prescribed inhaled corticosteroids was 74% (range 21-99%). Disappointingly, nearly 60% of patients had suboptimal adherence (<80%), despite being aware that adherence was being monitored. After the adherence monitoring intervention, the study identified four patient groups based on adherence and asthma symptoms post intervention. These four groups were a. STRA group (18%) with good adherence but persistent poor control, b. good adherence and improved symptom control group (24%), the monitoring most likely improved adherence and resulted in improved asthma symptoms, c. over-treated group (26%), who had sub-optimal adherence but good control, suggesting that the patient was being over-treated with high dose inhaled corticosteroids, d. poor asthma symptom control group with sub-optimal adherence (32%) in whom the adherence monitoring intervention made no difference to their adherence. This study demonstrated the utility of objective monitoring of inhaled corticosteroids to measure adherence and distinguish difficult asthmatics from severe therapy resistant asthmatics.

FeNO suppression testing is an alternative strategy for measuring inhaled corticosteroid adherence. It has been used in adults as a marker of clinical response to treatment with inhaled corticosteroids (33). In FeNO suppression testing FeNO levels are measured before and after a period of directly observed therapy (DOT) with inhaled corticosteroids. Heaney et al., used remote DOT and FeNO suppression to assess adherence. Study participants had one week of remotely monitored directly observed therapy, in which high dose inhaled corticosteroids were administered daily. The FeNO suppression test was positive if a 42% decrease in FeNO was observed (34). Two hundred and one people in the study completed the test and over half (N=130) had positive suppression tests (33). FeNO suppression is a useful alternative approach to using electronic monitoring devices, which are expensive, often lost and also require a longer period of monitoring.

Adherence monitoring is important, not only to prevent overprescribing, but to identify patients with DA. Furthermore, information from adherence monitoring can be used to motivate families and children to improve adherence and modify their behaviour. In cases where support from the multidisciplinary team, including psychologist is not successful at improving adherence, directly observed therapy of inhaled corticosteroids at school can be implemented and in cases with significant concern a social services referral may be considered (35). Patients that are steroid responsive but have persistent poor adherence despite intervention are called 'refractory difficult asthmatics' (36).

Assessment of Home Environment

As part of a child's initial PSA assessment at the Royal Brompton Hospital the respiratory clinical nurse specialist undertakes a home visit (27). A home visit enables a first-hand assessment of smoke exposure, allergen exposure (house dust mite, mould, pets) and available medication in the house. It also provides opportunity for the family to talk with the nurse about the impact of managing a child with asthma on the family, difficulties they are experiencing in doing so, and for the specialist nurse to give advice on minimising environ-

mental triggers. In addition, the nurse may contact the school nurse and primary care physician for information on medication prescriptions, asthma inhaler use at school, participation in school sports, school attendance and any social concerns about the family (35).

Assessment of Co-Morbidities

Co-morbid conditions can complicate asthma management and lead to overtreatment or worsening of asthma symptoms. Appropriate assessment and investigation for co-morbid conditions is vital. The most common co-morbidities are chronic allergic rhinosinusitis and gastro-oesophageal reflux.

Gastro-oesophageal Reflux Disease

Gastro-oesophageal reflux disease (GORD) is associated with chronic respiratory disease (37). Approximately 20 to 80% of asthmatic children have GORD (38). The lack of large longitudinal randomized controlled studies and the inconsistency in GORD definition used, makes it difficult to accurately estimate the prevalence of GORD in asthma, determine a causal relationship and determine the impact of GORD on asthma symptoms (38). In a small randomized controlled trial of lansoprazole in children with poorly controlled asthma and asymptomatic gastro-oesophageal reflux, no effect on asthma control was observed (39). The current evidence suggests there may be an association between GORD and asthma, but there is no evidence for gastro-oesophageal reflux treatment improving asthma symptoms or outcomes in paediatrics.

Rhinosinusitis

Approximately 60-80% of children with asthma have allergic rhinitis (40). Evidence from cohort studies in children with asthma shows an association between allergic rhinitis and poor asthma control, and that intranasal corticosteroids may have a beneficial effect on asthma control (41). It is important to optimally treat nasal symptoms to ensure they do not compromise asthma symptom control. However, there is limited evidence to support this relationship and randomized controlled trials of intranasal corticosteroids would be the best way of determining the relationship between allergic rhinitis symptoms and asthma symptoms.

Obesity

Epidemiological studies have demonstrated an association between obesity in childhood and asthma (42-45). Further evidence from meta-analysis of prospective cohort studies has shown a two-fold increase in the incidence of asthma in childhood if obese (42). Conversely, children with asthma are at an increased risk of obesity because of daily inhaled corticosteroids, and reduced activity levels because of concerns about triggering asthma symptoms (45, 46). The current evidence suggests that the relationship between asthma and obesity is bi-directional, and that asthma can cause obesity, but can also be triggered by obesity. The proposed underlying mechanisms for obesity associated asthma are complex and include altered systemic inflammation and metabolic dysregulation (47). In a paediatric asthma cohort study, a non-atopic Type 1 (Th1) helper driven endotype, was observed in obese asthmatic children (48). Obesity in asthmatic children is associated with worse asthma control, lung function, quality of life, increased risk of asthma exacerbations and reduced response to inhaled corticosteroids (49-52). Though obesity can worsen reflux symptoms, it is important to not overtreat, and consider that gastro-oesophageal reflux is more prevalent in obesity and may result in increased perceived asthma symptoms (53). Similarly, physical deconditioning in obesity due to lack of exercise, may also result in mislabeling of symptoms as asthma (54). A multi-disciplinary team input for children with severe asthma is important to reliably assess asthma symptoms, prevent obesity by encouraging physical activity and support children that are obese to lose weight.

Dysfunctional Breathing

Dysfunctional breathing is defined as recurrent or chronic changes in breathing pattern, which results in respiratory and non-respiratory symptoms, such as chest or throat tightness, shortness of breath, wheeze and anxiety (55). It is a blanket term that encompasses vocal cord dysfunction, breathing pattern disorder and hyperventilation syndrome (56). The Nijmegen Questionnaire (NQ) is a validated screening tool for dysfunctional breathing in adults, and is also used for screening in children, but has not been validated (57, 58). The prevalance of dysfunctional breathing was 5% in a cross-sectional survey of 203 hospital outpatient children with mild to moderate asthma, this was associated with worse asthma symptom control (59). An assessment by a specialist physiotherapist is useful to identify and treat breathing pattern disorders. Breathing exercises are recommended for adults, though the same exercises are age-adapted and used in children, there is limited available evidence in children (53). However, a cohort study of 169 children with asthma and dysfunctional breathing observed that a breathing exercise intervention had a significant improvement in asthma symptoms and a reduction in dysfunctional breathing screening score (60).

Psychosocial Factors

Co-existent psychosocial factors are associated with poor asthma symptom control. Psychosocial factors were associated with a quarter of asthma deaths identified in the National Review of Asthma Deaths (7). An estimated 25% of children with asthma have anxiety and or depression, these children have a higher rate of emergency department attendances for asthma symptoms (61). The healthcare beliefs of the child and their family, as well as symptom perception all influence adherence to prescribed treatment and engagement with healthcare intervention. Questionnaires can be used to provide a baseline understanding of impact of asthma on the child and their family. This includes the Asthma Control Test (ACT), Childhood Asthma Control Test and Paediatric Asthma Quality of Life Questionnaire (PAQLQ) for children aged 12 years and older (12, 13, 62). Ultimately, input from a paediatric psychologist is invaluable in identifying psychosocial concerns and working together with the family and the rest of the team to support the family in the form of tailored support, involvement of social services and schools if necessary and individual counselling (35).

After the comprehensive multidisciplinary assessment children with modifiable factors are classified as DA. Those with persistent symptoms despite good treatment adherence and absence of modifiable factors are classified as STRA. The latter group require further investigation. It is important to remember that even children categorized as DA, may have persistent symptoms after modifiable factors have been addressed and if so, should be managed as STRA.

Testing of Children with Severe Therapy Resistant Asthma

Blood Tests

If not previously done earlier in the assessment the following blood tests should be undertaken: serum Total IgE, blood eosinophil count, Vitamin D level and specific IgE antibody measurements to common aeroallergens (cat, dog, mixed grass, mixed tree, *Penicillium notatum, Alternaria alternata, Aspergillus fumigatus*), food allergens (peanut, egg, milk). Total serum IgE and eosinophil count are particularly important for guiding choice of biologic.

Sputum Induction

Sputum induction enables a safe, non-invasive assessment of airway cellularity and airway inflammatory phenotype. Sputum induction is a GINA recommended assessment in adults and adolescents with severe asthma (4). Sputum eosinophil guided management in adults with a confirmed asthma diagnosis has been shown to be beneficial.

A Cochrane systematic review of sputum eosinophil guided asthma therapy included 5 randomised controlled trials in adults and showed a significant reduction in frequency of asthma exacerbations in adults OR 0.57 (95% CI 0.38 to 0.86). The review included one randomized controlled trial in 54 children that did demonstrate a reduction in exacerbations but did not reach statistical significance 0.76 (95% CI 0.54 to 1.04) P=0.09. Currently, there is insufficient evidence for sputum eosinophil guided management in children. However, sputum is an important tool for assessing inflammatory phenotype; this will be increasingly important when choosing immune directed biologic therapy.

Fibreoptic Bronchoscopy

Bronchoscopy is an invasive procedure that enables assessment of inflammation and airway remodeling. It also enables direct assessment of airway structure, sampling of lower airway infection, inflammatory cells using lavage and airway histology assessment by biopsy. The results obtained can be used to immunophenotype the airways.

Assessment of Response to Systemic Corticosteroids and Additional Add on Treatments

Approximately 20% of children with severe asthma have true STRA, these children require further assessment to determine pathological phenotype and determine if systemic corticosteroid responsive (32). This can be assessed by giving a single dose of intramuscular triamcinolone and measuring change in asthma symptoms, spirometry (FEV_1, FEV_1/FVC Ratio), BDR, FeNO, sputum and bronchoalveolar lavage eosinophils before and 1 month after treatment (63). The pattern of response to treatment with systemic corticosteroid may help to decide the optimal add-on therapy for the individual child. For example, a significant improvement in exhaled nitric oxide after triamcinolone has been shown to be a predictor of positive response to omalizumab (63).

Long-Acting Muscarinic Antagonists for STRA

Tiotropium bromide is a long-acting muscarinic antagonist which causes bronchodilation by non-specific blocking of the muscarinic acetylcholine receptor, therefore inhibiting smooth muscle contraction as well as mucus secretion (64). A Phase-III double blind randomized controlled trial of tiotropium bromide plus maintenance ICS treatment in children over 12 years old with severe asthma showed a numerical improvement in FEV_1, but this was not statistically significant (48). A similar phase-III trial of once-daily Respimat (tiotropium) 5 μg in children 6-11 years old with severe asthma on ICS plus one or more other treatments showed a statistically significant increase in FEV_1. The addition of tiotropium may limit loss of asthma control and improve FEV_1. Tiotropium use is therefore recommended as an additional treatment by ATS/ERS Severe Asthma Management Guidelines for children with severe asthma on Step 4/5 of GINA treatment (65, 66). However, it should be given as a trial of therapy, and if no improvement in symptoms or lung function is seen in the individual child, then it should be stopped.

Biologics

Severe asthma in children is a heterogeneous disease. The advent of the recent biologic era, and "omics" analyses (such as transcriptomics, proteomics, metabolomics, genomics) and increasing understanding of disease inflammatory pathways and mechanisms, has highlighted the need to develop biomarker guided severe asthma therapy. The majority of children have allergic eosinophilic asthma, mediated by a T2 helper (Th2) molecular pathway; including IgE production and inflammatory mediator cytokines IL-4, IL-5 and IL-13. Biomarkers that indicate activation of this pathway include FeNO, blood eosinophils and broncho-alveolar lavage or sputum eosinophilia. Biologics are an add on (immune targeted) treatment therapy for severe asthma that reduce asthma exacerbations. The current biologics used in children target the Th2 pathway mediating allergic asthma.

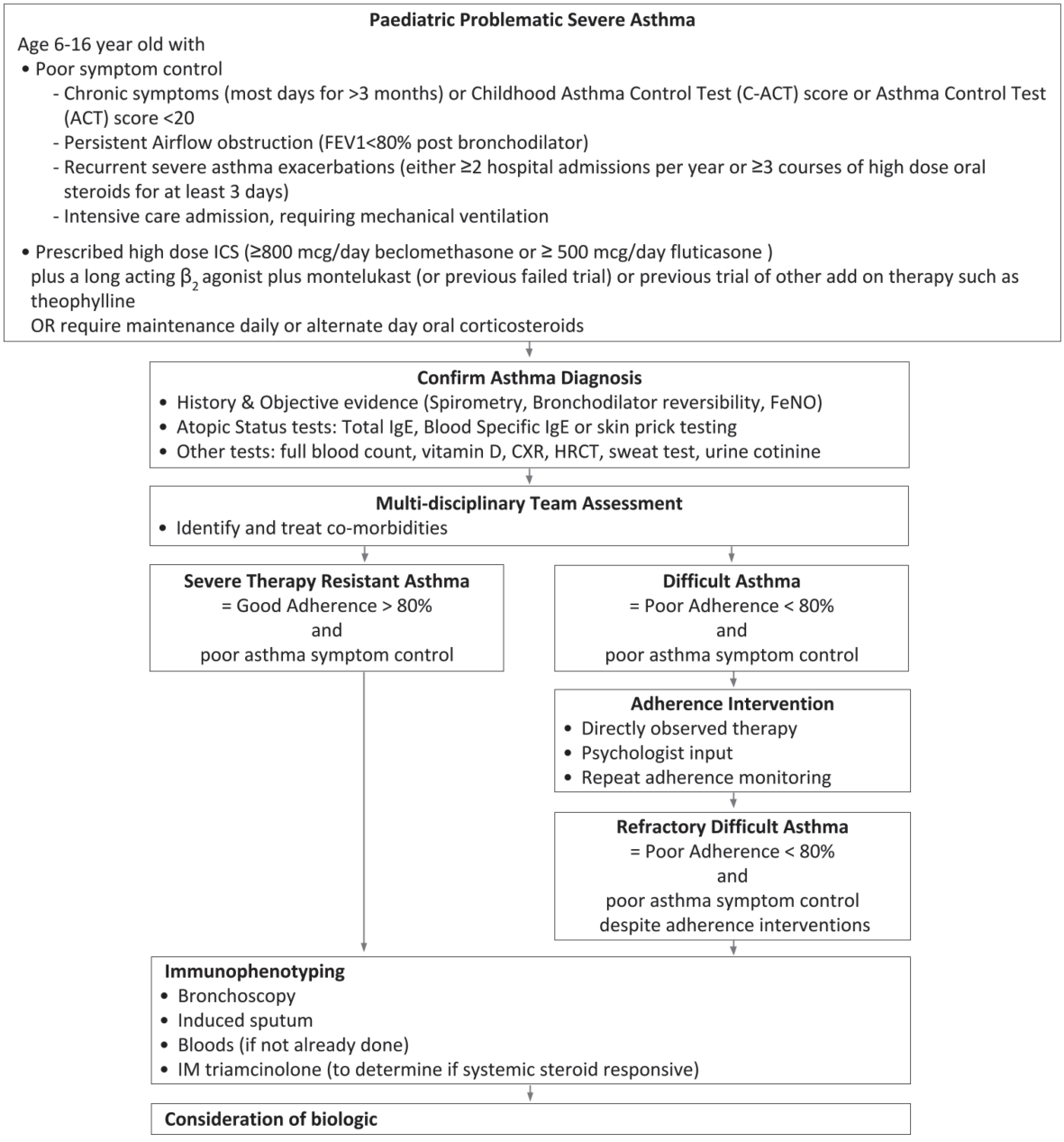

Figure 1. Paediatric Problematic Severe Asthma Assessment Pathway.

The 2019 GINA guidelines suggest considering biologics at step 5 (4). Though evidence is limited, and refractory DA group have not been a focus of clinical trials; children with refractory DA and unresponsive to strategies to improve treatment adherence, should also be considered for biologics, in order to prevent a fatal attack.

Omalizumab

Omalizumab (Xolair) is a subcutaneous injection licensed by European Medicines Agency (EMA) and US Federal Drug Administration (FDA) for use in children with allergic STRA (67). It is an anti-immunoglobulin E (IgE) humanised monoclonal antibody. It binds to circulating free IgE, which

consequently neutralises IgE and results in down-regulation of IgE receptors on mast cells, basophils and dendritic cells and results in reduced Th2 inflammatory cytokines (68). Three randomised controlled trials in children (N=1381) have compared omalizumab with placebo in children with PSA. The main effect of omalizumab is a reduction in exacerbations. The number of asthma exacerbations was lower for children on omalizumab (26.7% vs. 40.6%) (69). However, one-third of children do not have a clinical response to omalizumab and a further third of patients are not eligible for omalizumab under the current prescribing guidance because of the narrow serum IgE range recommended by the manufacturer. Of note, its efficacy has not been tested in children with true STRA, only in those with PSA.

Mepolizumab, Reslizumab and Benralizumab

Mepolizumab is an anti IL-5 monoclonal antibody subcutaenous injection. It prevents IL-5 mediated eosinophil activation and reduces eosinophil survival (70). It is licensed in Europe for use as an add on treatment in children with severe asthma aged 6-16 years old. Although adult data shows that mepolizumab is both safe and efficacious, there have been a limited number of children in clinical trials and no efficicacy trials in children aged 6-11 years old. However, a non-randomised open label trial of mepolizumab in children aged 6-11 has demonstrated similar pharmacodynamics to adults and that it is safe to use in this age group (71). Current prescribing guidelines include severe asthma with poor symptom control despite good adherence to treatment, blood eosinophil count ≥300/μl and 4 or more courses of oral steroids in last 12 months or daily oral steroids for last 6 months. The MENSA and DREAM trials showed a reduction in exacerbations and blood eosinophil count, but included only 34 children aged 12 to 17 years old (72).

Reslizumab, like mepolizumab is also an anti-IL-5 monoclonal antibody, but is less attractive for children as it has to be administered using the intravenous route. It is not yet licenced for use in children in Europe. Benralizumab is an IL-5Rα receptor antibody and also does not currently have a licence for children in Europe. There have not been any clinical efficacy trials in children for either drug. Anti-IL-5 or anti-IL-5Rα treatment is recommended for adults with blood eosinophils ≥300/μL in GINA guidelines if on step 4/5 of asthma management and persistent poor asthma control (73). The latest ATS/ERS severe asthma guidelines advise a lower eosinophil threshold of ≥150/μL for adults (49). The literature has focussed on blood eosinophils as a marker, rather than other markers of eosinophilic inflammation (such as sputum eosinophils) due to ease of obtaining a blood eosinophil count. Randomised clinical trials in children are needed to help determine IL-5 monoclonal antibody treatment thresholds, utility of biomarkers and efficacy.

Conclusion

Approximately 2-5% of all children with asthma have PSA. It is essential that children with PSA are referred to a specialist paediatric respiratory centre for confirmation of diagnosis, a step-wise multidisciplinary team assessment and ongoing management, including consideration of additional steroid sparing treatments, such as biologics. Firstly, the diagnosis of asthma must be confirmed, using history and objective tests. In tandem a multidisciplinary assessment is essential to assess for co-morbidities, identify modifiable environmental factors and assess treatment adherence. 50-80% of children have poor treatment adherence and a smaller number have true STRA. Biologics are a new and important additional treatment, however clinical trials to date in children have been limited in size, the only randomized controlled trials of biologics in children of all ages have been with omalizumab, but even then, none have included only children with STRA. However, only two-thirds of children with STRA are eligible for omalizumab because of the narrow serum IgE range in which it can be prescribed. This means additional biologics are important and needed for children. However, trials of efficacy are lacking and biomarkers of eosinophilic inflammation and markers to predict

response for different paediatric phenotypes are currently lacking but are urgently needed to guide future choice of biologic.

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Narrative Review
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Respiratory Virus Infections in Asthma: Research Developments and Therapeutic Advances

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Abstract
In this review, we discuss the latest developments in research pertaining to virus-induced asthma exacerbations and consider recent advances in treatment options. Asthma is a chronic disease of the airways that continues to impose a substantial clinical burden worldwide. Asthma exacerbations, characterised by an acute deterioration in respiratory symptoms and airflow obstruction, are associated with significant morbidity and mortality. These episodes are most commonly triggered by respiratory virus infections. The mechanisms underlying the pathogenesis of virus-induced exacerbations have been the focus of extensive biomedical research. Developing a robust understanding of the interplay between respiratory viruses and the host immune response will be critical for developing more efficacious, targeted therapies for exacerbations. **Conclusion.** There has been significant recent progress in our understanding of the mechanisms underlying virus-induced airway inflammation in asthma and these advances will underpin the development of future clinical therapies.

Key Words: Asthma ▪ Respiratory Viruses ▪ Interferon ▪ Therapy.

Introduction

Asthma is a heterogeneous condition characterised by chronic airway inflammation, variable expiratory airflow limitation, bronchial hyperresponsiveness and variable symptoms including shortness of breath, wheeze and cough (1). As per the Global Asthma Network's Global Asthma Report 2018, the Global Burden of Disease study estimated that approximately 339 million people were affected by asthma in 2016; and asthma was ranked the 16th leading cause of years lived with a disability and 23rd leading cause of premature mortality (2). Common asthma clinical phenotypes include: early-onset allergic asthma, characterised by elevated levels of specific immunoglobulin E (IgE) and type 2 helper T cell (T_H2) cytokines, and associated with other allergic diseases and responsiveness to corticosteroid therapy; late-onset eosinophilic asthma, defined by an eosinophilia that may be refractory to corticosteroids and responsive to anti-interleukin(IL)-5 therapy; exercise-induced asthma, which is associated with activation of mast cells, T_H2 cytokines and cysteinyl leukotrienes; neutrophilic asthma, characterised by sputum neutrophilia and activation of T_H17 immune pathways; and obesity-related asthma, which primarily affects women and in which there is little airway inflammation (3). It has recently been suggested that using multiplex serum assays can help to classify asthma according to the underlying triggers and thus distinguish between allergen- and virus-triggered asthma, which in turn may assist in tailoring personalised therapies (4).

A loss of asthma control or a worsening of symptoms is termed an 'exacerbation', the severity of which is determined by a combination of the clinical history and changes in lung function parameters (5). Exacerbations are associated with not only significantly worse health-related quality of life, but also with marked increases in healthcare-related expenditure for both patients and healthcare systems (6, 7). To prevent and treat exacerbations successfully, understanding their aetiology is critical. Respiratory viral infections are the most common cause of asthma exacerbations and thus a robust understanding of the pathogenic mechanisms underlying virus-induced exacerbations will be vital to facilitate the development of new treatment strategies (8).

This article provides an overview of recent advances in our understanding of the role of respiratory viruses in asthma exacerbations and highlights the latest studies on potential therapies.

Aetiology of Viral Exacerbations of Asthma

Respiratory viral infections remain the most common cause of asthma exacerbations. Studies have previously demonstrated that 80-85% of exacerbations among school aged children with asthma are caused by respiratory viruses (9). Similarly, among adults with asthma, symptomatic colds have been associated with 80% of exacerbations (10) and respiratory viruses have been detected in the sputum of 76% of asthma exacerbation cases in adults (11).

Among the most common causes of viral asthma exacerbations are rhinoviruses (RV), respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses (PIV), coronaviruses, adenoviruses and human metapneumovirus (Table 1). The number of viral respiratory infections during early life, rather than the specific viral pathogen, appears to be associated with an increased risk of developing asthma during later life (12). RSV bronchiolitis/lower respiratory infection in early life is particularly associated with the development of recurrent wheeze and asthma in later childhood (13), although interestingly recent evidence suggests that RSV prevention during infancy may not necessarily confer protection against subsequent development of asthma (14). RV infection is the most common trigger of viral asthma exacerbations in adults (15). Wheezing with RV co-infection in early life is an even greater risk factor for subsequent asthma (odds ratio 9.8) than wheezing with RSV co-infection (odds ratio 2.6) (16). Recent advances in the diagnosis of respiratory virus infections include the development of a chip containing microarrayed proteins/peptides from RV,

Table 1. Summary of Common Respiratory Viruses that Cause Asthma Exacerbations (18-24)

Virus	Family	Genome	Cell receptor / binding sites	Reference
Rhinoviruses	Picornaviridae	Single-stranded RNA	Major group serotypes (including both A & B species): Intercellular adhesion molecule-1 (ICAM-1)	(18)
			Minor group serotypes (all A species): Low-density-lipoprotein receptor (LDLR)	
			Group C species: Cadherin-related family member 3 (CDHR3)	
Respiratory syncytial virus	Paramyxoviridae	Single-stranded RNA	Nucleolin	(19)
Influenza viruses	Orthomyxoviridae	Single-stranded RNA	Sialic acids	(20)
Parainfluenza viruses	Paramyxoviridae	Single-stranded RNA	Sialic acids	(21)
Coronaviruses	Coronaviridae	Single-stranded RNA	Strain-specific; e.g. SARS-CoV binds to angiotensin-converting enzyme 2 (ACE2), MERS-CoV binds to dipeptidyl peptidase 4 (DPP4)	(22)
Adenovirus	Adenoviridae	Double-stranded DNA	Cell surface integrins αvβ3 and αvβ5	(23)
Human metapneumovirus	Paramyxoviridae	Single-stranded RNA	Cell surface integrin αvβ1	(24)

which has enabled the rapid diagnosis of RV-A and RV-C (far less commonly, RV-B) in serology from a paediatric population with RV-induced wheeze (17).

Deficiencies in Immunological Responses to Viral Infections in Asthma

Evidence for a deficiency in interferon (IFN) responses – Type I IFNs such as IFN-α and IFN-β play an important role in antiviral immune responses. Several studies have demonstrated that the host IFN response to rhinovirus infection is deficient in both asthma and chronic obstructive pulmonary disease (COPD) (25, 26). Initial evidence for this came from work undertaken by Wark and colleagues, in which primary bronchial epithelial cells (BECs) sampled from people with asthma and healthy controls were infected with RV-16. There was a significant impairment in speed of onset as well as magnitude of IFN-β mRNA expression and IFN-β protein production in RV-infected cells from people with asthma compared to RV-infected cells from controls. There was significantly enhanced viral replication in cells from people with asthma compared to healthy controls, but following administration of exogenous IFN-β, viral replication rates were significantly reduced (27). Further work by Contoli and colleagues showed that people with asthma also exhibit deficient production of type III IFNs such as IFN-λ1 and IFN-λ2/3. Relative to healthy controls, there was significant deficiency in induction of these IFNs in BECs and bronchoalveolar lavage (BAL) cells from people with asthma following RV infection (28). Furthermore, IFN-λ production in response to *ex vivo* RV infection of BAL cells sampled before experimental RV infection *in vivo* was strongly related to virus load, airway inflammation and symptom severity during the subsequent *in vivo* infection, strongly implicating IFN deficiency with increased exacerbation severity (28). These findings were reinforced by subsequent studies demonstrating that primary BECs from people with asthma exhibit significantly less IFN-λ production compared to healthy controls following RV-1B infection (29). RV-induced type I and type III IFN responses are significantly diminished in asthmatic subjects irrespective of their atopic status (30). This IFN deficiency is also seen in severe therapy resistant asthmatic children, whose BECs (compared to controls) exhibit a higher viral load that negatively correlates with IFN-β and IFN-λ mRNA levels (31).

Recently, an *in vivo* study demonstrated that there is deficient IFN-α and IFN-β protein expression by BECs in people with asthma compared to controls at baseline. Lower levels of epithelial IFN-α and IFN-β expression were correlated with more severe cold symptoms, worse airway hyperresponsiveness and greater reductions in forced expiratory volume in 1 second (FEV_1) following RV infection. Furthermore, the study showed that deficiency in IFN induction among people with asthma was not associated with deficient pattern recognition receptor (PRR) expression at baseline (32).

It has also been suggested that type I IFN deficiency seen in asthma may depend on the degree of asthma control at the time of sampling and perhaps also on asthma subtype. In a study of adult patients with sub-optimal asthma control, peripheral blood mononuclear cells (PBMCs) were stimulated with RV-1B *in vitro* and IFN responses were measured: PBMCs from patients with neutrophilic asthma exhibited significantly impaired IFN-α production compared to PBMCs from those with eosinophilic asthma and paucigranulocytic asthma, though inhaled steroid dose was also an independent predictor of decreased IFN-α protein production (33).

Measurement of cytokine responses to RV infection of PBMCs from children may offer a way to predict the likely course of asthma in later life. In a recent study, PBMCs from children were stimulated with RV and cytokine production was subsequently measured. The study identified that distinct cytokine immunophenotypes were associated with different clinical trajectories. The greatest risk of developing asthma in later life and of experiencing severe asthma exacerbations was associated with the lowest levels of IFN induction

and high levels of proinflammatory cytokine production in response to RV stimulation of PBMCs. Conversely, the lowest risk of asthma was seen in the group who exhibited moderate IFN induction and the highest induction of proinflammatory cytokines following RV infection of PBMCs (34). These findings have been reinforced by another recent study which showed that neonates whose cord blood cells did not produce any type I or type III IFNs in response to polyinosinic: polycytidylic acid (poly I:C) stimulation had a significantly increased risk of developing febrile lower respiratory infection in the first year of life, as well as a four-fold increased risk of persistent wheeze at age five years, compared to those neonates who produced at least one type I or type III IFN at birth, suggesting an important role for developmental regulation of type I and type III IFN production in subsequent risk of asthma development (35). Novel immunophenotyping in this manner may enable better understanding of the mechanisms underlying virus-induced allergic airways disease in children and in later life.

Further ways of predicting short-term asthma exacerbation risk have been highlighted in a recent transcriptome analysis. This demonstrated that children with high nasal 'type 2 inflammation' module expression and low nasal 'type 1 IFN response' module expression exhibit a faster time to asthma exacerbation; and the most significant risk of exacerbations in the short-term is among those in whom there is an elevated ratio of 'type 2 inflammation' to 'type 1 IFN response'. Diminished IFN signalling at baseline may permit increased viral replication to occur, which in turn promotes asthma exacerbation with enhanced IFN responses during exacerbation, consequent upon increased virus load (36).

Studies not reporting a deficiency in IFN responses in asthma – An extensive discussion regarding studies not reporting a deficiency in type I and/or type III IFN responses in asthma can be found in a recent review (25). Here, we highlight studies not previously described in that review. In a study by Moskwa and colleagues in which human BECs were infected with either PIV type 3 (PIV3) (multiplicity of infection (MOI) 0.1) or RV-1B (MOI 0.1), viral replication rates in BECs were not different between people with asthma or controls for either virus and no differences were observed in IFN-α, IFN-β or IFN-λ1 mRNA or IFN- λ1 protein production between the two groups (37). It is possible that the lack of difference in IFN induction between asthmatic and non-asthmatic cells was related to the MOI used: MOI 0.1 was used in this study whereas the study by Wark and colleagues that demonstrated significant impairment in IFN-β induction in RV-infected asthmatic BECs used MOI 2 (27). Additionally, the BECs in the study by Moskwa and colleagues were derived from a mixture of atopic (N=6) and non-atopic (N=4) asthmatics, whereas most studies reporting impairment in IFN induction in virus-infected asthmatic BECs have used BECs derived from only atopic asthmatics. Indeed, sub-group analysis of the asthma group showed that, compared to atopic asthmatic cells, non-atopic asthmatic cells exhibited lower IFN-λ1 protein and lower IFN-α/IFN-β mRNA in response to PIV3; and lower IFN-β mRNA in response to RV (37). Subject numbers were small (10 asthmatics and 9 healthy controls) and the majority of asthma subjects had mild asthma (37).

A recent study showed that BECs from children who have asthma and obstructive lung function exhibited greater RSV-induced type I and type III IFN expression compared to BECs from children with asthma who had a non-obstructive lung function picture and compared to healthy controls (38). However, this study only assessed IFN responses late at 96hrs after RSV infection (MOI 0.5); thus it is possible that deficient IFN induction early following infection may have enabled greater viral replication during the early phase of infection, which secondarily induced an exaggerated IFN response later during infection. This interpretation is supported by the observation of around 10 times higher RSV copy numbers at 96 hrs in the BECs from children with asthma and obstructive lung function, compared to the healthy control subjects.

Reconciling the discrepancy in the evidence base relating to the IFN response in viral asthma exacerbations will require further study. However,

possible explanations for the seemingly divergent evidence base include that different studies have used different virus strains, different MOI and have studied different time points during infection in subjects with differing severities of asthma and different cell culture conditions (39). Concurrent treatments being taken by study participants may also affect the degree of IFN expression: for example, it has recently been shown that use of inhaled corticosteroid therapy suppresses IFN production in virus-induced exacerbations of COPD, resulting in increased pulmonary bacterial load (40). All these factors, and very likely others as well, may result in heterogeneity in the degree of IFN response that has been measured.

Airway Inflammation in Response to Viral Infections in Asthma

Eosinophils – The role of eosinophils in promoting airways inflammation in patients with allergic asthma is well established. The antiviral effects of eosinophils have previously been attributed to secreted effector proteins including eosinophil-derived neurotoxin (41), which exhibits RNAse activity (42). Human eosinophils treated with a nitric oxide synthase inhibitor have a diminished antiviral effect against PIV, suggesting that nitric oxide likely plays a role in eosinophils' anti-infective activity (43). Additionally, transfer of eosinophils from MyD88-deficient mice to wildtype mice results in the recipient mice having higher RSV loads, suggesting that the antiviral properties of eosinophils may be contingent on the toll-like receptor (TLR) -7/MyD88 signalling pathway (44). Transferring eosinophils from allergen-sensitised mice into influenza A virus-infected mice has been shown to reduce virus load and improve lung compliance in the infected mice, suggesting that eosinophils may confer a protective effect against respiratory viral infections (45). An *in vivo* study has demonstrated the ability of pulmonary eosinophils to internalise and inactivate influenza virus in mice and the ability of blood eosinophils from healthy human subjects to similarly capture and inactivate RSV and influenza virus. This capability of eosinophils was found to be significantly diminished in patients with asthma and greater asthma severity was associated with a greater reduction in virus capture by eosinophils (46). It should be noted that anti-IL-5 therapies, which target IL-5 or the IL-5 receptor and thus reduce eosinophil numbers/activation, have been shown to reduce the frequency of clinically significant exacerbations in people with asthma and eosinophilia (47). Relevant clinical trials are discussed later in this review.

IL-33 – Use of novel mucosal sampling techniques such as nasosorption and bronchosorption have demonstrated that there is marked local induction of IFNs and T_H2 responses in the upper and lower airways of asthma patients following RV infection (48). RV infection induces IL-33 and a T_H2 cytokine response in the airways of asthma patients, with IL-33 levels correlating with IL-5 and IL-13 levels. Blockade of the IL-33 receptor abolishes RV-induced T_H2 cytokine production by human T cells and type 2 innate lymphoid cells, suggesting that IL-33 plays an integral functional role in promoting the T_H2 inflammatory response to RV infection in asthma (49). This is further reinforced by a study in sensitised mice which has shown that IL-33 suppresses innate antiviral responses and adaptive T_H1 responses in influenza-induced exacerbations, thus promoting enhanced airway inflammation (50). Administration of anti-IL-33 therapy in sensitised mice that have been exposed to RV decreases the T_H2 immune response throughout the course of the subsequent disease, decreases exacerbation severity and promotes expression of IFN-α, IFN-λ and IFN-γ. However, it has no effect on airway smooth muscle remodelling during the chronic phase of disease (51).

It has been suggested that the cellular immune response to IL-33 following RV infection differs between people with asthma and healthy individuals. A study in which PBMCs from subjects with allergic asthma and healthy controls were co-stimulated with IL-33 and RV showed that while IL-33 augmented RV-induced IL-5 and IL-13 production in PBMCs from asthma patients, it had no effect on IL-5 and IL-13 production in PBMCs from healthy

controls. Additionally, IL-33 promoted innate lymphoid cell production of IL-13 in PMBCs from asthma patients but promoted natural killer cell production of IFN-γ in PBMCs from controls (52).

Transcriptome network analysis of nasal and blood samples from children with virus-induced exacerbations has also shown that there is upregulation of the *IL33* gene among this group compared to those who have had non-viral exacerbations (36). A study of virus-induced asthma exacerbation using wildtype and IL-1β knockout mice has shown that IL-33 expression upon virus inoculation is contingent on the IL-1β signalling pathway, with the latter also shown to promote expression of neutrophil chemoattractants and the mucin MUC5AC (53).

IL-25 – IL-25 regulates T_H2 responses and has been shown to contribute to the pathogenesis of allergic asthma (54). More recently, its role in RV-induced asthma exacerbations has been elucidated. Compared to RV infection of BECs from healthy controls, RV infection of BECs from people with asthma *in vitro* resulted in significantly greater *IL25* mRNA expression at 8 hours post-infection and greater IL-25 protein expression at 24 hours post-infection (55). In the same report, human experimental RV infection showed that relative to baseline, subjects with asthma exhibited a significant increase in IL-25 protein concentrations in nasal mucosal lining fluid following RV infection *in vivo*, whereas healthy controls exhibited a more modest increase. Additionally, in mouse models of virus-induced exacerbation of allergic airways disease, RV-induced IL-25 expression was associated with enhanced T_H2 cytokine induction, while IL-25 receptor blockade resulted in a reduction in T_H2 responses and MUC5AC production. Thus IL-25 plays a key role in the pathogenesis of virus-induced allergic airways inflammation (55).

IL-18 – RV infection has recently been shown to induce IL-18 in humans *in vivo*, both among people with asthma and healthy controls. Regardless of asthma status, individuals with a low baseline nasal IL-18 level developed more severe colds following RV infection than those individuals with a high baseline nasal IL-18 level, suggesting that airway mucosal IL-18 levels prior to RV infection may serve as an important predictor of symptom severity following RV inoculation. This suggests that IL-18 may have a protective effect against RV infection of the respiratory tract, although the mechanisms underlying this effect remain unclear (56).

Neutrophil Extracellular Traps (NETs)

Neutrophils have been shown to exert a protective effect against respiratory tract infections caused by influenza virus, as evidenced by enhanced viral replication in influenza-infected neutrophil-depleted mice (57). Studies have previously demonstrated that RV infection in people with asthma is associated with a significant increase in the number of bronchial epithelial and subepithelial neutrophils; and that the bronchial neutrophil count is positively associated with the RV virus load (58). While the process of neutrophil recruitment, phagocytosis and release of toxic granules is well established, recent attention has shifted to how neutrophil extracellular traps (NETs) contribute to inflammation in respiratory disease (59).

NETs, which comprise double-stranded DNA (dsDNA), histones and granular proteins, are released by neutrophils into the extracellular space and stimulate a T_H2 immune response (60). NETs have been identified in bronchial biopsies from people with mild allergic asthma (61) and high concentrations of NETs in the sputum are associated with more severe asthma, characterised by low Asthma Control Test scores, mucus hypersecretion and frequent use of oral corticosteroids (62). NET formation may be stimulated by neutrophil exposure to virus: RSV fusion protein has been shown to interact with TLR4 on neutrophils and thereby trigger NET formation (63). A role for NETs in driving the severity of RV-induced asthma exacerbations has recently been demonstrated: RV infection of subjects with asthma resulted in significant release of host dsDNA in nasal lavage samples and this correlated with symptom severity and bronchial concentrations of the T_H2 cytokines IL-5 and IL-13. Reducing the level of dsDNA in the airways of RV-infected allergic mice using DNase

and inhibition of NET formation using an elastase inhibitor resulted in a decrease in bronchoalveolar lavage fluid eosinophils, lymphocytes and mucins as well as lung T_H2 lymphocytes and cytokines with a reduction in RV-exacerbated airway hyperresponsiveness and airway inflammation (64).

Therapeutic Developments

Given the significant health and socioeconomic impact of viral asthma exacerbations, robust prevention and treatment strategies are needed.

Potential biomarkers – In a study of patients with asthma who were steroid-naïve, serum IFN-γ-induced protein 10 (IP-10; also known as CXCL10) levels were shown to be higher among those with virus-induced asthma compared to those with non-viral exacerbations; serum IP-10 levels had a sensitivity of 95% and specificity of 70% for virus-induced asthma (65). An observational study has demonstrated that serum IgG concentration taken at the time of hospital admission among patients with confirmed virus-induced severe asthma exacerbations is significantly lower than the admission serum IgG concentration among those admitted with non-viral asthma exacerbations. Low serum IgG at the time of admission is associated with longer duration of oral corticosteroids and hospital stay. However, statistical analysis demonstrated that serum IgG concentration had poor specificity and modest sensitivity for predicting the severity of asthma exacerbations, suggesting that the clinical utility of measuring this requires further study (66).

Vaccinations – There remains limited evidence on how efficacious respiratory virus vaccinations are at decreasing the rate of viral exacerbations in asthma patients. A Cochrane systematic review of influenza vaccination in people with asthma demonstrated that there was no reduction in influenza-induced asthma exacerbations among children; however, children who had been vaccinated had better symptom scores during influenza-positive weeks. Studies in adults did not contribute useful data due to very low levels of confirmed influenza infection (67). However, in a subsequent systematic review and meta-analysis which included robust quasi-experimental and epidemiological studies in addition to randomised controlled trials, it was shown that influenza vaccination may prevent up to 78% of asthma attacks that necessitate unscheduled emergency hospital visits (68). Although there is evidence to suggest that administration of a recombinant VP0 capsid protein RV vaccination in mice promotes more effective RV clearance following RV challenge (69), the considerable heterogeneity in human RV strains means that developing clinically effective vaccinations remains a challenge and there are still no approved vaccinations against RV for use in humans.

Antiviral agents –Targeting the cellular receptors of viruses offers a potential therapeutic target. RV major group serotypes bind to intercellular adhesion molecule 1 (ICAM-1), which is a leukocyte adhesion molecule (70) that serves as a receptor for RV (71). Blocking such cellular adhesion molecules, which otherwise regulate immune cell function and migration, may offer a way of reducing inflammation (72). Blockade of ICAM-1 by a novel anti-human ICAM-1 antibody (14C11) has been shown to reduce airway inflammation following infection with RV-14 and RV-16 in a mouse model (73). The use of recombinant soluble ICAM-1 (tremacamra) in humans has been shown to reduce the severity of RV infection symptoms compared placebo control (74), but the high frequency of dosing required prevented further clinical development. Various drugs targeting the RV capsid have been studied: intranasal pirodavir was found to have a significant antiviral effect but did not provide clinical improvement in cold symptoms (75); oral pleconaril reduced the duration of cold symptoms by one day compared to placebo (76), but was associated with bleeding in women taking oral contraceptives and was rejected by the US Food and Drug Administration (77); and vapendavir had an antiviral effect but did not provide an improvement in lung function or reduction in asthma exacerbations in patients with RV infection (78).

Macrolide antibiotics – Studies have suggested that macrolides have a beneficial effect when used in the treatment of asthma exacerbations:

for example, telithromycin has been shown to significantly reduce exacerbation symptoms (79). However, the mechanisms underlying the beneficial effects of macrolides remain unclear. Studies have suggested that macrolides may exhibit antiviral properties by restoring deficient IFN responses and attention has turned to utilising this in the treatment of viral asthma exacerbations. Azithromycin has been shown to augment RV-1B and RV-16 induced IFN production in human BECs while decreasing RV replication in vitro; this effect was not seen with telithromycin (80). In a randomised controlled study in adult patients with asthma, azithromycin treatment did not result in a significant improvement in asthma symptoms scores compared to placebo; however, interpretation of this study is difficult as for every patient randomised, more than 10 were excluded for having already received antibiotics (81). Among children aged 1 – 3 years, azithromycin has been shown to significantly reduce the duration of asthma-like symptoms compared to placebo, with reductions being greater among those who were started on therapy before the sixth day of symptoms (82). Among children with a history of recurrent severe lower respiratory tract infections (LRTIs), early use of azithromycin during a LRTI significantly reduces the risk of a clinically severe LRTI developing (83). Several novel macrolides that are derivatives of azithromycin, erythromycin and oleandomycin have also been shown to augment IFN responses in BECs that have been infected with RV *in vitro* and one has been shown to have anti-viral activity in BECs from people with asthma (84).

Inhaled IFN therapy – Given the evidence for IFN deficiency in the pathogenesis of virus-induced asthma exacerbations, a randomised double-blind placebo-controlled study was undertaken to evaluate the clinical effectiveness of administering a 14-day regimen of inhaled IFN-β therapy following onset of cold symptoms in asthma patients. Although the therapy did not have a significant effect on the primary endpoint of patient-reported symptoms, sub-group analysis showed that it did improve reported symptoms among people with moderate to severe asthma. Additionally, treatment with inhaled IFN-β was associated with an improvement in morning peak expiratory flow and enhanced expression of antiviral biomarkers such as serum CXCL10 and sputum CXCL10, Mx1 and OAS1 (85). Thus, inhaled IFN-β therapy may ameliorate virus-induced symptoms in those with moderate to severe asthma, but further confirmatory studies are needed. In a recent *in vitro* study, monocyte-derived macrophages, alveolar macrophages and primary BECs from healthy controls and COPD patients were infected with influenza virus either before or after administration of exogenous IFN-β. Cell infection was significantly reduced in all three cell types when IFN-β was administered prior to influenza infection, but this effect was not present when administered post-influenza infection. This suggests that prophylactic IFN-β therapy may be of utility in reducing respiratory tract viral infections; additional *in vivo* clinical studies are needed to evaluate this further (86).

Anti-IgE therapy – The clinical utility of anti-IgE therapies in severe allergic asthma is well-established (87). The potential for such therapies to treat and shorten the duration of virus-induced exacerbations has shown promise. In a paediatric allergic asthma population, the use of omalizumab as a treatment adjunct for virus-induced exacerbations has been shown to reduce the duration of RV infections by approximately 1 day, reduce peak viral shedding and decrease the frequency of RV illnesses (88). In another paediatric study, omalizumab significantly decreased the severity of RV-induced asthma exacerbations, even among patients who started with a poorer baseline disease activity (89). The beneficial effect of omalizumab in treating virus-induced exacerbations may be due to its effect on IFN-α production. In a recent study, PBMCs and plasmacytoid dendritic cells (pDCs) were isolated from children with recurrent asthma exacerbations, both before and during treatment with omalizumab (90). The PBMCs and pDCs were stimulated *ex vivo* with either RV or influenza virus and IFN-α protein production was subsequently measured. Relative to pre-omalizumab treatment, omalizumab treatment (in the presence of IgE cross-linking) significantly increased IFN-α

production by 2.06-fold in RV-treated PBMCs, 1.57-fold in influenza-treated PBMCs and 4.15-fold in RV-treated pDCs (90). This reinforces previously reported data showing that omalizumab improved peripheral blood mononuclear cell generation of IFN-α in response to RV among children with asthma; and that, among the omalizumab group, greater IFN-α increases were associated with significantly fewer exacerbations (91).

Anti-IL-5 therapy – The clinical utility of anti-IL-5 therapy in the prevention of exacerbations in severe eosinophilic asthma has been established (92). Given the potential role of eosinophils in virus-induced exacerbations, a recent study has investigated the therapeutic value of using anti-IL-5 therapy in mild asthma to attenuate the eosinophil-mediated immune response to RV-16 infection. While administration of mepolizumab did diminish baseline blood and tended to diminish sputum eosinophil counts, it did not cause a significant improvement in lung function or in fractional exhaled nitric oxide after RV challenge, nor did it prevent eosinophil activation following RV-16 inoculation (93). Benralizumab has been shown to reduce annual asthma exacerbation rates and improve prebronchodilator FEV_1 among patients with severe, uncontrolled asthma and eosinophilia (94). Reslizumab has been shown to reduce frequency of asthma exacerbations among patients with inadequately controlled, moderate-to-severe asthma and eosinophilia (95). While virology testing was not performed in these latter two studies, it is likely that many of the asthma exacerbations would have been viral in origin.

Anti-IL-13 therapy – Lebrikizumab, a humanized IgG_4 anti-IL-13 monoclonal antibody, has been shown to reduce exacerbation frequency by 60% and improve lung function in patients with moderate to severe asthma who have high levels of serum periostin (96). A subsequent study has demonstrated that lebrikizumab reduces exacerbation rates among patients with uncontrolled asthma who have high periostin levels or high blood eosinophil counts, but these effects were not consistently observed in the replicate study (97). In contrast, a phase 2b study of tralokinumab, a fully human IgG_4 anti-IL-13 monoclonal antibody, showed that it did not significantly reduce exacerbation rates in patients with severe asthma compared to placebo, although there may be an improvement in exacerbation frequency and symptom control in those with high serum periostin and high serum dipeptidyl peptidase 4 (DPP4) levels (98). A recent study has also shown that tralokinumab has an inconsistent beneficial effect on annualised asthma exacerbation rates among severe asthmatics with high baseline fractional exhaled nitric oxide (FeNO; a non-invasive surrogate marker of airway inflammation in patients with asthma (99)) (100).

Anti-IL-4/13 therapy – Dupilumab, a monoclonal antibody against the α subunit of the IL-4 receptor which inhibits both the IL-4 and the IL-13 signalling pathways, reduces exacerbation rates and improves lung function in moderate to severe eosinophilic asthmatics when long-acting β_2-agonist/steroid combination therapy is withdrawn (101). Recently, a study among patients with uncontrolled asthma demonstrated that administration of dupilumab results in a significant reduction in annualised rates of severe asthma exacerbations and a significant increase in FEV_1 compared to matched placebo controls, with the greatest clinical benefits being observed among those with a higher baseline blood eosinophil count (102).

PRR Antagonism – PRRs activated by RV infection include TLRs such as TLR3, melanoma differentiation-associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I) (103, 104). Based on pre-clinical model data, it was hypothesised that blockade of TLR3 and its downstream signalling pathways may offer a novel way in which to diminish the inflammatory effect of RV infection in asthma. In a randomised controlled study in asthma patients, the use of CNTO3157, an inhibitory anti-TLR3 monoclonal antibody, was found to be ineffective at protecting against symptoms or decreases in FEV_1 following RV inoculation and its use was associated with a greater number of post-inoculation moderate and severe exacerbations compared to those receiving placebo (105). TLR3 inhibition has been shown to de-

crease IFN-λ production in airway epithelial cells that have been stimulated with the TLR3 agonist poly I:C (106). It is therefore possible that TLR3 blockade with CNTO3157 dampened the IFN response following RV inoculation, thus permitting increased viral replication which in turn triggered more exacerbations.

CXCR2 receptor antagonists – Neutrophil activation and migration are regulated by the CXCR2 receptor. The potential to block neutrophil inflammatory pathways by using CXCR2 receptor antagonists in asthma has been under investigation for some time. In a randomised placebo-controlled trial involving patients with severe asthma, the CXCR2 antagonist SCH527123 was shown to significantly reduce sputum neutrophil count and tended (P=0.05) to reduce mild exacerbation frequency (107). However, in a separate trial using the CXCR2 antagonist AZD5069 in patients with uncontrolled persistent asthma, there was no effect on the frequency of severe exacerbations (108). The therapeutic value of utilising such drugs to target NETopathic inflammation in virus-induced asthma exacerbations remains to be seen (109).

Conclusions

Respiratory virus infections play an integral role in the pathogenesis of asthma exacerbations. In recent years, studies have shed further light on the potential mechanisms underlying the interactions between respiratory viruses and the immune system. There is substantial evidence suggesting deficiencies in the host immune response may predispose many asthma patients to virus-induced exacerbations. There have been several recent advances in our understanding of the mechanisms underlying virus-induced airway inflammation in asthma, including growing evidence around the role of pro-inflammatory cytokines such as IL-33, NETs, eosinophils and the interaction between viruses and bacterial infections. Developing a more robust understanding of these mechanisms will be critical for developing novel and more efficacious therapies to prevent and treat virus-induced asthma exacerbations.

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Effect of Farming on Asthma

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Abstract
In this review, we discuss an immunobiology model of farm exposure towards the protective effect of asthma. Unraveling the protective effect of farming exposure could help develop novel strategies to prevent asthma. Asthma is a chronic airway inflammation that causes coughing, wheezing, chest tightness or shortness of breath. The reasons for the increase in the prevalence of asthma worldwide is still unclear but has been hypothesized to be attributable to westernization/urbanization of rural regions thus resulting in the loss of rural farming environmental. In this review we discuss the effect of the environmental factors, specifically farming, on the risk of asthma in children. Here, we will summarize the main findings of 27 studies related to 11 different cohorts. Several studies have shown preventive effect of traditional farming on the prevalence and incidence of asthma in childhood. Furthermore, consumption of unpasteurized cow's milk, exposure to farm animals as well as fodder have been shown to have a protective effect on asthma. The precise mechanism of the protective effect is still unclear. There are assumptions, that maternal/childhood exposures to farm animals result in higher microbial exposures through which the protective effect might be mediated. Also, consumption of unpasteurized milk (when consumed during pregnancy by mother or early childhood by children) can modulate cytokine production patterns which could be responsible for the observed protective effect. **Conclusion.** This review provides evidence of the protective effect of farming environment i.e., exposure to farm animals, their fodder as well as consumption of unpasteurized cow's milk suggesting that novel strategies could be developed to prevent asthma.

Key Words: Asthma Epidemiology ▪ Childhood ▪ Farming ▪ Farm Milk ▪ Stables.

Introduction

Asthma is a chronic airway inflammation that causes coughing, wheezing, chest tightness or shortness of breath. It is a complex disease and is likely to be determined by multiple intrinsic and extrinsic factors. The intrinsic factors comprise gender, race, genetic predisposition and atopy whereas the extrinsic factors involves environmental influences like air pollution, allergens and smoking (1). Globally, asthma is ranked 16th among the leading causes of years lived with disability and 28th among the leading causes of burden of disease (2). The prevalence of asthma varies with global geographical position, with higher prevalence observed in Australasia, Europe and North America, as well as in parts of Latin America with lowest prevalence observed in the Indian subcontinent, Asia-Pacific, Eastern Mediterranean, and Northern and Eastern Europe (2). Based on the survey of the respiratory disease statistics from the European Union (EU)-28, 5.9% of the adult population reported that they suffered from asthma, with higher prevalence reported in women (6.6%) than in men (5.2%) (3). Similarly, according to the Centers for Disease Control and Prevention (CDC) from the United States, 1 out of 13 people have asthma which is more common in children (8.4%) than in adults (7.7%). Furthermore, children aged 5–11 years (9.6%) and 12–17 years (10.5%) were more affected than children aged 0–4 years (3.8%) (4). Moreover, the annual

Table 1. Overview of the Studies Assessing the Effect of Farm Environment on Asthma

Studies*	Size of the study	Exposure	Main findings
Riedler et al. (9).	Cross-sectional study mostly rural area in Austria; N=2001.	Living on farm.	Austrian children living on a farm have less asthma than children from a non-farming environment.
von Ehrenstein et al. (10).	Cross-sectional study in two Bavarian districts with extensive farming activity; N=10163.	Living on farm.	Farmers' children had lower prevalence of asthma. The protective effect was stronger for children whose families were running the farm on a full-time basis as compared with families with part-time farming activity.
Perkin et al. (11).	Cross-sectional study in the rural county of Shropshire, England; N=4767 (Stage 1) and N=879 (Stage 2).	Farm-related exposure and endotoxin.	Farmers' children had significantly less current asthma symptoms compared to nonfarming children. Children drinking unpasteurized milk were producing higher levels of IFN-γ.
Midodzi et al. (12).	Longitudinal study consisting of rural farming, rural non-farming and non-rural environments children; N=13524.	Living on farm.	The 2-year cumulative incidence of asthma was higher in children living in non-rural environment. Children living in a non-rural environment with parental history of asthma had an increased risk of asthma incidence when compared with children living in rural non-farming environment.
Illi et al. (13).	Rural regions of Austria, Germany and Switzerland; N=79888 (Phase I) and N=8419 (Phase II).	Farm-related exposures (contact with animals, stay in animal sheds, contact with animal feed, presence during parental farming activities, stay in barn or fodder storage room, and consumption of cow's milk produced on the farm) and IgE measurements.	Children living on a farm were at significantly reduced risk of asthma compared with nonfarm children. Traditional farming (i.e., with cows and cultivation) was protective against asthma.
Riedler et al. (14).	Cross-sectional study in rural areas of Austria, Germany, and Switzerland; N=812.	Timing, frequency, and intensity of children's exposure to stables and farm and pet animals, mothers' activity on the farm, duration of breastfeeding, timing of consumption of home-grown food and farm milk, vaccinations, avoidance of allergens, dust samples and serum IgE measurements.	Exposure of children younger than 1 year, compared with those aged 1–5 years, to stables and consumption of farm milk was associated with lower frequencies of asthma. Protection against development of asthma was independent from effect on atopic sensitization. Continual long-term exposure to stables until age 5 years was associated with the lowest frequencies of asthma.
Ege et al. (15).	Cross-sectional study from rural areas in 5 European countries; N=8263.	Farm-related exposures, allergen-specific IgE, RNA expression of CD14 and Toll-like receptor genes, and dust from children's mattresses was collected for microbial components.	Protective effect was found for keeping pig, farm milk consumption, frequent stay in animal sheds, child's involvement in haying and usage of silage. Protective factors were related with higher expression levels of genes of the innate immunity. Fungal extracellular polysaccharides was associated with protective effect for asthma.
Brunekreef et al. (16).	The study consist of data from 28 countries; N=194794 (exposure to farm animals) and N=194598 (maternal exposure to farm animals during pregnancy).	Early life exposure to farm animals.	A positive association was found between early exposure to farm animals and the prevalence of symptoms of asthma.

Table 1 (continued)

Studies*	Size of the study	Exposure	Main findings
Dong et al. (17).	Cross-sectional study in 3 cities in Liaoning province, China; N=16789.	Exposures to common indoor allergens.	Positive association was found between exposure to pets and farm animals with asthma.
Hugg et al. (18).	Cross-sectional study in towns of Finnish-Russian border; N=1093.	Exposures to pet and farm animals.	Increased risk of asthma in the urban children exposed to farm animals during early life.
Waser et al. (19).	Cross-sectional study from rural areas in 5 European countries; N=14893.	Dietary component and allergen-specific IgE.	Farm milk consumption ever in life showed an inverse association with asthma. Protective effect of other farm products except vegetables or fruits on asthma was found.
Brick et al. (20).	Birth cohort from the rural areas of Austria, Germany, Switzerland, Finland and France; nested case-control study of 35 asthmatic and 49 nonasthmatic children.	Fatty acid (FA) composition of unprocessed farm milk and industrially processed milk.	Consumption of unprocessed farm milk compared with shop milk was associated with protective effect of asthma. Part of the effect was explained by the higher levels of omega-3 polyunsaturated FAs.
Pfefferle et al. (21)*.	Birth cohort from the rural areas of Austria, Germany, Switzerland, Finland and France; N=625.	Maternal exposure to farming activities and farm dairy products during pregnancy. Outcome: Cytokine production in cord blood of children.	Maternal exposure to farming activities and farm dairy products during pregnancy modulated cytokine production patterns of offspring at birth.
Douwes et al. (22).	Rural cross-section study from New Zealand; N=1899.	Current, early and prenatal farm-related exposures.	Farmers' children had a lower incidence of asthma symptoms. Maternal exposure during pregnancy to farm animals and grain and/or hay reduced the risk of asthma symptoms.
Schaub et al. (23)*.	Birth cohort in rural southern Germany; N=82.	Maternal farming exposures. Outcome: Treg cells in cord blood stimulated with microbial stimulus and cytokines.	Farm exposures during pregnancy increase the number and function of cord blood Treg cells associated with lower TH2 cytokine secretion and lymphocyte proliferation on innate exposure.
Braun-Fahrländer et al. (24)*.	Rural areas of Germany, Austria or Switzerland; N=812.	Endotoxins levels in the bedding of children. Outcome: Asthma and cytokines production.	Endotoxin levels from the child's mattress were inversely related to the asthma. Cytokine production by leukocytes was inversely related to the endotoxin level in the bedding.
Schram et al. (25)*.	Cross-sectional study from rural areas in 5 European countries; N=478.	Living on farm. Outcome: To assess the levels of bacterial endotoxin, mould beta(1, 3)-glucans and fungal extracellular polysaccharides in house dust of children.	Farm children are not only consistently exposed to higher levels of endotoxin, but also to higher levels of mould components.
Stein et al. (26).	60 Amish and Hutterite children.	Levels of allergens, endotoxins and assessing the microbiome composition of indoor dust samples.	The prevalence of asthma was low in Amish children. Median endotoxin levels in Amish house dust was high. There was differences between the proportions, phenotypes, and functions of innate immune cells between both the two groups of children.
Ege et al. (27)*.	Cross-sectional studies; N=6843 (Parsifal) and N=9668 (Gabriela).	Living on farm and microbial exposure. Outcome: Asthma, screening for bacterial DNA and assess the levels and bacterial and fungal taxa in house dust of children.	In both studies, children who lived on farms had lower prevalence of asthma. The diversity of microbial exposure was inversely related to the risk of asthma.
Karvonen et al. (28).	Birth cohort in Finland; N=410.	Environmental microbial from house dust samples.	A score for the total quantity of microbial exposure was significantly (inverted-U shape) associated with asthma incidence.

Table 1 (continued)

Studies*	Size of the study	Exposure	Main findings
Birzele et al. (29)*.	Cross-sectional; N=86.	Bacterial community composition in mattress dust and nasal samples. Outcome: Asthma and farm exposure.	Farm exposure was positively associated with bacterial diversity in mattress dust samples. Asthma was inversely associated with bacterial diversity in mattress dust.
Kirjavainen et al. (30).	Birth cohorts (farm and non-farm); N=415.	House dust microbiota.	In the children grown up in non-farm homes, the risk of asthma decreases as the similarity of their home bacterial microbiota composition to that of farm homes increases.
Lluis et al. (45)*.	Munich Metropolitan area, Germany; N=200.	Genotypes of ten single nucleotide polymorphisms (SNP) covering the 17q21 locus. Outcome: Gene expression in 17q21 region and T-cell subsets in cord blood as well as gene expression of ORMDL3 in early and adult life.	The results suggest an association of 17q21 SNPs with ORMDL3, GSDMA expression and IL-17 secretion early in life.
Loss et al. (46)*.	Rural regions of Austria, Finland, France, Germany and Switzerland; N=983.	Genotyped SNPs in ORMDL3 and GSDMB genes at 17q21 and farming environmental exposure. Outcome: Asthma and wheeze.	17q21 locus relates to episodes of acute airway obstruction common to both transient wheeze and asthma.
Eder et al. (47).	Cross-sectional study in rural areas of Austria and Germany; N=609.	Genotyped SNPs in the TLR2 and TLR4 genes.	Genetic variation in TLR2 gene is a major determinant of the susceptibility to asthma in children of farmers.
Loss et al. (48)*.	Rural regions of Austria, Finland, France, Germany and Switzerland; N=938 (blood samples at birth) and 752 (year 1).	Framing environmental and nutritional exposure as well as polymorphisms in innate receptor genes. Outcome: mRNA expression of the Toll-like receptor (TLR) 1 through TLR9 and CD14.	Gene expression of innate immunity receptors in cord blood was higher in neonates of farmers. Unpasteurized farm milk consumption during the first year of life showed the strongest association with mRNA expression at year 1. Modification of the association between farm milk consumption and CD14 gene expression by the SNP CD14/C-1721T was not found.
Ege et al. (49)*.	Cross-sectional study from rural areas in 5 European countries; N=8263 and subsample (N=322) used for gene expression.	Maternal and child's exposure to microbial and farming environment. Outcome: Asthma and gene expression of TLR2, TLR4 and CD14.	Gene expression of receptors of innate immunity was strongly determined by maternal exposure to stable during pregnancy. Each additional farm animal species increased the gene expression of TLR2, TLR4 and CD14.

*If outcome other than asthma was used for the discussion of the findings in the study.

economic cost of asthma (from 2008-2013) in the United States, which includes medical costs as well as loss of work and school days, was reported more than $81.9 billion in US (5).

The reasons for the increase in the prevalence of asthma worldwide is still unclear but has been hypothesized to be attributable to westernization/urbanization of rural regions thus resulting in the loss of rural farming environment. Several epidemiological as well as intervention studies have been conducted to understand the effect of the environmental factors on the risk of asthma (6-8). In this review we discuss the effect of the environmental factors, specifically farming environment, on the risk of asthma in children. We would like to state that there are many more farm studies that have been performed. Since this review is not a systematic review we have selected those studies which describes the important aspects of farm upbringing. Table 1 summarizes the main findings of 27 studies related to 11 different cohorts which are discussed in this review.

The aim of this review is to summarize the effect of the farming environment on the risk of asthma in children.

Farming Environment

Epidemiological studies have shown protective effect of traditional farming on the prevalence and incidence of asthma in childhood. There are several studies that have looked at the effect of farming (exposures) with the risk of asthma (8). The study from Riedler et al. conducted in rural area in Austria (with a high proportion of farmers) observed a low prevalence in the farm children compared to the nonfarm children (1.1% vs. 3.9%) (9). In the study from von Ehrenstein et al. conducted in two rural Bavarian districts in Germany showed similar low prevalence of the doctor diagnosed asthma in the farm children (3.4%) compared to nonfarm children (6.4%) (10). Perkin and Strachan compared rural nonfarming and farmers' children and showed that farmer's children when compared to the rural nonfarm children had significantly less current asthma symptoms (adjusted odds ratio (aOR) 95% confidence interval (95% CI): 0.67 (0.49-0.91)) (11). Not only higher prevalence but also higher incidence of asthma was determined in a longitudinal study from Canada. The study by Midodzi et al. consisting of 13524 asthma-free children at baseline (aged 0-11) were surveyed and the 2-year cumulative incidence of asthma was reported to be 2.3% among children living in farm, 5.3% rural nonfarm and 5.7% non-rural environments (12). The study further showed that the children living in the farm environment had a reduced risk of asthma compared with children from rural nonfarm environment (OR (95% CI): 0.22 (0.07-0.74)).

The relationship between being raised or living on a farm and its protective effect on developing asthma has been investigated intensively. However, the possible causal mechanism between this associations are still not understood. Several studies are carried out to find which aspect of farm characteristics i.e., animal barns, exposure to straw and consumption of farm milk, can explain the protective effect of farming on the risk of asthma. The study from von Ehrenstein et al. showed a protective effect of full-time and part-time farming activity for asthma (part-time: OR (95% CI): 0.80 (0.37-0.83) and full-time: 0.45 (0.26-0.78)) suggesting a dose response effect (10). The study further showed the consumption of whole milk was higher among the farmer's offspring than among other children. Furthermore, the study by Illi et al. using the data of the GABRIEL Advanced Studies showed similar low prevalence of asthma in the farm children (11.4% Phase I and 14.1% Phase II) compared to the nonfarm children, with the exposed nonfarm children (i.e.; children not living on a farm but regularly exposed to stables, barns, or cow's milk produced on a farm) having intermediate prevalence (15.8% Phase I and 20.0 Phase II) (13). In the analysis adjusted for study centers, and potential confounders (family atopy, >2 siblings, sex, maternal smoking in pregnancy, and parental education), a protective effect for asthma was observed (aOR (95% CI): 0.68 (0.59-0.78)). This study further stratified the analysis based on types of farms and the exposure of a child to specific farm characteristics. The authors first identified 3 types of farms based on a latent class analysis. A protective effect of the third type of farming (comprised of dairy cows and breed cattle combined with cultivation mostly of grain and corn) compared to the first type of farming (comprised of pigs, poultry or horses combined with cultivation of grain and feeding of grain shed) within the farm children was observed for asthma (aOR (95% CI): 0.79 (0.65-0.95)). Furthermore, exploring the child's exposure to farm characteristics showed protective effect of i) having contact with cow (aOR (95% CI): 0.74 (0.62-0.89)), ii) staying with cow (aOR (95% CI): 0.79 (0.65-0.95)), iii) contact with straw (aOR (95% CI): 0.79 (0.66-0.95)), iv) present with parents during manuring (aOR (95% CI): 0.65 (0.47-0.90)) and v) consumption of farm milk (aOR (95% CI): 0.77 (0.66-0.90)) (13). The study by Riedler et al. showed similar protective effect of farming on the risk of asthma (aOR (95% CI): 0.30 (0.15-0.61)) and further looked at the exposures to several farming environmental factors (14). A substantial protection against developing asthma was seen only in the children exposed to stables and farm in the 1st year of life (aOR (95% CI): 0.14 (0.04-0.48)). Protection was also related

to the continuing exposure after the first year of life to the stable compared to children who had no exposure to the stables in their first 5 years of life (aOR (95% CI): 0.09 (0.01-0.75)) (14). Ege et al. likewise looked at the association between several farming exposures and the risk of asthma showing protective effect of i) keeping pig (OR (95% CI): 0.57 (0.38-0.86)), ii) consumption of farm milk (OR (95% CI): 0.77 (0.60-0.99)), iii) frequent stay in the animal sheds (OR (95% CI): 0.71 (0.54-0.95)) and iv) child's involvement in haying (OR (95% CI): 0.56 (0.38-0.81)) (15).

In the study by Brunekreef et al. i.e., the Phase Three of the International Study of Asthma and Allergies in Childhood (ISAAC), which was carried out in 6- to 7-year-old children in urban populations across the world does not confirm the protective effects of farming environment on asthma. Further, stratifying by gross national income, the association between farm animal exposure in the first year of life with asthma was much stronger in the non-affluent (1.27 (1.12-1.44)) than in the affluent countries (0.96 (0.86–1.08)). Similar effect was observed in the analyses using exposure to farm animals during pregnancy. The reason for this could be that the children enrolled in this study were from urban or semi-urban areas rather than from rural areas having occasional rather than frequent or continuous exposure to farm animals in pregnancy and the first year of life (16). Similarly, the study by Dong et al. found positive association between exposure to cats, dogs, and farm animals with asthma. This study was carried out in 3 cities in Liaoning province, China (17). Another study by Hugg et al. conducted in the towns of Imatra in Finland and Svetogorsk in Russia also showed increased risk of asthma in the urban children exposed to farm animals during early life (18).

Looking at the relationship between farm-produced products and asthma, the study by Waser et al. similar to others showed protective effect of farm milk consumption with asthma (aOR (95% CI): 0.74 (0.61-0.88)) and other farm products except vegetables or fruits (19). To further asses the protective effect of unpasteurized cow's milk consumption on asthma Brick et al. used the data of a birth cohort to determine whether the differences in the fatty acid (FA) composition of the unpasteurized farm milk and the industrially processed milk contributed to this effect. The study showed that the consumption of unpasteurized farm milk compared to the shop milk was associated with a protective effect on asthma (aOR for consumption at the age of 4 years (95% CI): 0.26 (0.10-0.67)). The author further showed that the part of the effect could be explained by the higher fat content of the farm milk, especially the higher levels of ω-3 polyunsaturated FAs (aOR (95% CI): 0.29 (0.11-0.81)) (20). The study by Perking and Strachan investigated the relationship between farming environment and developing allergic problem and suggests that one of the possible mechanism for this observed protective effect may be through greater consumption of farm or unpasteurized milk. However, to note that the effect of frequent consumption of farm or unpasteurized milk on the risk of asthma did not show statistical significant association (OR (95% CI): 0.73 (0.53-1.02)) in the study (11). This non statistical significant association could be due to confounding factors in the farming environment that could be correlated with the farm-milk consumption. Pfefferle et al. investigated the associations between maternal farm exposures and cytokine levels in cord blood using the data of the Protection Against Allergy: Study in Rural Environments (PASTURE) birth cohort concluding that the maternal exposure to farming activities and farm dairy products during pregnancy modulate cytokine production patterns of offspring (21).

The study by Douwes et al. further looked at the mother's farm exposure toward the protective effect of children's asthma (22). The study showed that farmer's children had lower incidence of asthma symptoms compared to the nonfarm children. Current and maternal exposure during pregnancy to animals and/or grain and hay reduced the risk of asthma symptoms. A combination of prenatal and current exposure was strongly associated with asthma medication (OR (95% CI): 0.50 (0.30–0.82)) and asthma ever (OR (95% CI): 0.50 (0.33–0.76)) in the study. The study concluded that

prenatal exposure may contribute to the low prevalence of asthma, hay fever and eczema in farmers' children, but continued exposure may be required to maintain optimal protection.

Immunological Studies of Farm Exposures

Schaub et al. showed that the farm exposures during pregnancy increase the number and function of cord blood Treg cells associated with lower T_H2 cytokine secretion and lymphocyte proliferation on innate exposure speculating that maternal farm exposure might reflect a natural model of immunotherapy (23). It has been suggested that the enhanced exposure to endotoxin (bacterial lipopolysaccharide (LPS)) is an important protective factor of farm environments. Braun-Fahrländer et al. assessed the levels of endotoxin in the bedding used by the farming and nonfarming children and examined it's relation to asthma. The results of the study showed that the endotoxin levels in samples of dust from the child's mattress were inversely related to the occurrence of asthma (24). Schram et al. evaluated the levels and determinants of bacterial endotoxin, mould beta (1, 3)-glucans and fungal extracellular polysaccharides (EPS) in the house dust of farm children, Steiner school children and reference children (25). The authors concluded that the farm children are not only consistently exposed to higher levels of endotoxin, but also to higher levels of mould components.

The above studies show that children who are grown up in the traditional farm environments are protected from developing asthma. This "farm effect" can be mainly hypothesized by the child's early life interaction with farm animals, in particular cows, and their milk products and microbes. The study from Stein et al. further demonstrated that this protective effect is mediated through innate immune pathways (26). The study used the data from the Amish and Hutterite school children living on farms in the United States. The prevalence of asthma in the Amish farm children was lower (5.2%) compared to the Hutterite farm children (21.3%). The authors further showed that microbial burden and composition differ between the Amish and Hutterite home environments. Although, there is a remarkable genetic similarity between Amish and Hutterite children, the opposite effects of their house dust on the airway responses and inflammation as observed in the mouse models, suggest that environmental exposures confer strong protection from asthma among the Amish by engaging innate immune responses, whereas the lack of such exposures and/or the presence of unidentified risk exposures promotes asthma risk among the Hutterites. Ege et al. used the data of the PARSIFAL and GABRIELA studies and concluded that children living on farms were exposed to a wider range of microbes than were the children from the reference group and the range of microbial exposure was inversely associated with asthma (27). Furthermore, a study by Karvonen et al. looked at the microbial exposures as a predictor of asthma using a birth cohort (28). The study showed that the associations of single microbial markers with risk of asthma was nonsignificant. However, the total quantity of microbial exposure (sum of indicators for fungi, Gram-positive bacteria, and Gram-negative bacteria) showed significant inverted-U-shaped association with incidence of asthma (28). The highest risk was found at medium levels and the lowest risk at the highest level. Birzele et al. showed that the farm exposure was positively associated with bacterial diversity found in the mattress dust samples (as determined by richness and Shannon index) of 86 school age children (29). In this study, asthma was inversely associated with richness and Shannon index in mattress dust. A recent study by Kirjavainen et al. modeled differences in house dust microbiota composition between farm and non-farm homes of Finnish birth cohorts, LUKAS1 and LUKAS2, showing that in children who grow up in non-farm homes, asthma risk decreases as the similarity of their home bacterial microbiota composition to that of farm homes increases. This effect was replicated in the GABRIELA Study. The authors conclude that the indoor dust microbiota composition appears to be a potential modifiable target for asthma prevention (30).

Genetic Factors Related to Asthma

In this section we will only focus on gene-environment interaction i.e., how few genes predispose towards an effect of farm-environment on asthma.

Asthma is not only influenced by environmental factors but also has genetic determinants associated with it. Twin studies have estimated the heritability of asthma to range between 35 to 95% (31, 32). Largescale genome-wide association studies have identified almost 30 loci that are associated with asthma (32-41). The analyses of the UK Biobank data consisting of 380503 study participants indicated that the asthma associated risk variants collectively explains 2.5% of the variation in disease (42). Many of these loci map to the genes that are involved in immune responses or transcription factors that mediate the immune responses. Single nucleotide polymorphisms (SNP) on chromosome 17q21 have been most robustly associated with childhood asthma and asthma in children exposed to environmental tobacco smoke. Two genes ORMDL3 and GSDM have emerged as the most likely candidate genes for asthma (43, 44). Lluis et al. investigated the relationship between the polymorphisms and the mRNA expression of 17q21 locus genes and their influence on the T-cell subsets in the cord blood of the children from the rural areas showing an association of 17q21 polymorphisms with ORMDL3 and GSDMA expression, as well as the secretion of IL-17 early in life. These results imply a functional role of the 17q21 locus affecting T-cell development during immune maturation (45). Further to test the environmental determinants of infections and wheeze in the first year of life, potential modifications of these associations by SNPs at ORMDL3 (rs8076131) and GSDMB (rs7216389, rs2290400) genes at 17q21, and the implications for different trajectories of wheeze using the data of the PASTURE birth cohort was conducted (46). The findings of the study suggest that the chromosome17q21 locus relates to episodes of acute airway obstruction which is common to both transient wheeze and asthma. The authors further suggests that the asthma risk alleles are the ones susceptible to the environmental influences. This gene–environment interaction revealed that the same genotype constitutes genetic risk and also allows for environmental protection, thereby providing options for prospective prevention strategies (46). Eder et al. used the data of the ALEX study to access if the polymorphisms in genes encoding TLRs might modulate the protective effects observed in farming populations. The carrier of a T allele in TLR2/−16934 among farmers' children compared to the children with AA genotype were significantly less likely to have a diagnosis of asthma (3% vs 13%, P=0.012) and current asthma symptoms (3% vs 16%, P=0.004), suggesting that the genetic variation in TLR2 gene is a major determinant of the susceptibility to asthma and allergies in children of farmers (47). In another study by Loss et al. the authors sought to determine the environmental and nutritional exposures associated with the gene expression of innate immunity receptors during pregnancy and the first year of a child's life using the data of the PASTURE birth cohort (48). Gene expression of innate immunity receptors in cord blood was overall higher in neonates of farmers (TLR7 and TLR8). Moreover, the unpasteurized farm milk consumption during the first year of life showed the strongest association with mRNA expression at year 1 (TLR4 and TLR6). Ege et al. sought to investigate the role of maternal exposures to environments rich in microbes for development of asthma in the innate immune system in offspring. The gene expression of receptors of innate immunity was strongly determined by maternal exposure to stables during pregnancy. With each additional farm animal species increased the expression of TLR2, TLR4 and CD14 (49). Thus farming and farming-related exposures, such as contact with animals and/or consumption of unpasteurized farm milk, that were previously reported to decrease the risk for allergic outcomes were associated with a change in gene expression of innate immunity receptors in early life. These observations support the possibility that the 17q21 locus as well as innate immunity receptors indirectly impact the risk of childhood onset asthma through its direct effect on early life wheezing illnesses or risk of allergic outcomes through gene-environment interaction.

Mechanisms of the Protective Effect of Farming on Asthma

The studies discussed above facilitated to identify a working model of the immunobiology of farm exposure as described in two important reviews (8, 50). This model suggests the contact with multiple animal species such as cows, pigs, poultry, horses, sheep and goats along with consumption of (unpasteurized) farm milk results in strong microbial exposure of women who carry out farming duties during pregnancy. This model emphasizes on the timing of the exposure i.e., during pregnancy and early life. This time exposure represents a biological window of opportunity for shaping subsequent immune reactivity. The results of the study by Pfefferle et al. (mentioned above) showed that the maternal exposure to farm animals is related to an increased production of interferon γ (INFγ) and TNFα from stimulated but unfractionated cord blood nuclear cells (21). Additional, maternal exposure to number of farm animals substantially enhanced the expression of Treg cell marker glucocorticoid-induced TNF receptor and secretion of IFNγ (Der p 1 and Ppg) by cord blood cells in response to the allergen and peptidoglycan (23). Likewise, the results from the PARSIFAL study regarding maternal exposure to number of farm animals with increase in gene expression of TLR2, TLR4 and CD14 in offspring additionally support this model (49). Finally, as shown in the study by Ege et al. the presence of many different farm animal species may increase the dose and diversity of the related microbial species which results in the protective effect (27).

Further, in the study by Pfefferle et al. the consumption of farm-produced butter during pregnancy has been shown to enhance the production of INFγ and TNFα from the unfractionated cord blood mononuclear cells. An additive effect was observed for the combined consumption of butter and unskimmed farm milk on INFγ and TNFα. These results suggest that consumption of dairy products originating from the unpasteurized cow's milk during pregnancy modulates cytokine production pattern in the newborn babies (21). Schaub et al. showed that the maternal intake of unprocessed farm milk was related to the epigenetic changes in the cord blood (23). The amount of Treg cell-specific demethylated region was higher in newborns of mothers consuming unprocessed cow's milk during pregnancy. Unprocessed cow's milk contains natural amount of milk fat and polyunsaturated- as well as conjugated fatty acids (CLA) (21). A randomized controlled trial in young healthy volunteers found decreased plasma immunoglobulin E levels and increased interleukin (IL)-10 levels after CLA supplementation. These results suggest an antiallergic potential of CLA in immune function (51), thus proposing the role of FA composition of the unprocessed cow's milk on the protective effect of asthma. Although the current evidence is weak to suggest a major role for microbial constituents of raw cow's milk, however, one cannot exclude the possibility that specific probiotics might be detected in future.

Conclusion

The evidence of the protective effect of farming environment i.e., exposure to farm animals, their fodder as well as consumption of unpasteurized cow's milk advocates that novel strategies to prevent asthma could be developed.

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SRL, ITÄ-Suomen Yliopisto, Interplan - Congress, Meeting & Event Management AG, INC, Ökosoziales Forum Oberösterreich, Imperial College London, WMA Kongress GmbH, University Hospital rechts der Isar, European Respiratory Society, HAL Allergie GmbH, PersonalGenomes.org, Nestlé Deutschland AG, Universitätsklinikum Aachen, SIAF - Swiss Institute of Allergy and Asthma Research, Deutsche Pharmazeutische Gesellschaft e. V.; Verein zur Förderung der Pneumologie am Krankenhaus Großhansdorf e.V., Pneumologie Developpement, Mondial Congress & Events GmbH & Co. KG,, Volkswagen Stiftung, Böhringer Ingelheim International GmbH, Hanson Wade Ltd., DSI Dansk Borneastma Center; author honoraria from Elsevier Ltd., Springer-Verlag GmbH, Schattauer GmbH, Georg Thieme Verlag, Springer Medizin Verlag GmbH; In addition, Dr. von Mutius has a patent Application number LU101064, Barn dust extract for the prevention and treatment of diseases pending, a patent Publication number EP2361632: Specific environmental bacteria for the protection from and/or the treatment of allergic, chronic inflammatory and/or autoimmune disorders with royalties paid to Protectimmun GmbH, a patent Publication number EP 1411977: Composition containing bacterial antigens used for the prophylaxis and the treatment of allergic diseases. licensed to Protectimmun GmbH, a patent Publication number EP1637147: Stable dust extract for allergy protection licensed to Protectimmun GmbH, and a patent Publication number EP 1964570: Pharmaceutical compound to protect against allergies and inflammatory diseases licensed to Protectimmun GmbH.

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The Airway Microbiome and Childhood Asthma – What Is the Link?

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Abstract
In this paper we propose to describe the available evidence from the literature on upper airway microbiome and its association with paediatric asthma and allergy. Recent advances in sequencing the bacterial 16S ribosomal RNA (16S rRNA) gene have enabled research into the complex communities of bacteria, known as the microbiome, that exist outside and inside the human body. Although the upper airways have long been recognised to host a microbiome, the lower airways are now known to contain a rich and diverse microbiome. This review first describes the microbiome of the upper and lower airways and then explores associations between the microbiome in the airways and bowel and asthma in children. The characteristics of the microbiome differ between nose and mouth, and between the mouth and bronchus in terms of burden and diversity of bacteria and in the predominant phyla present. There is a small literature which suggests that there are differences in the airway microbiome in early life between children who later have asthma compared to those who do not develop asthma. **Conclusion.** At the time of writing it is not clear whether the microbiome may cause childhood asthma, whether the conditions in the asthmatic airway encourage a different microbiome or whether a third factor confounds the relationship between airway microbiome and childhood asthma.

Key Words: Asthma ▪ Child ▪ Microbiome.

Introduction

Bacterial communities outside and inside the human body are complex, and the introduction of 16S rRNA gene sequencing, which amplifies and sequences the 16S rRNA gene in bacterial communities, has given some insight into the "microbiome" and its association with asthma, cystic fibrosis and broncho pulmonary dysplasia (1-3). This methodology has moved us from a position of very little objective microbiological data to one where we have an abundance of data. The "microbiome" describes microorganisms of all types i.e. commensal (healthy), symbiotic and pathogenic bacteria, viruses and fungi that share the human body (4). The human microbiome is found at different body sites, including those in regular communication with the external environment, i.e. the skin, the respiratory tract and the gastrointestinal tract.

One important leap forward in our understanding from this pioneering research has been the understanding that there is a healthy community of bacteria in the lower airways. Previously the lower respiratory tract was considered sterile since bacteria could not be cultured from sputum and bronchoalveolar lavage samples from healthy individuals using older methodologies. The bacteria which are associated with lower respiratory tract infection, e.g. *S pneumoniae* and *H influenzae*, are part of everyone's healthy commensal upper and lower airway microbiome.

Microbial colonization is multifactorial, and it starts at the time of birth and is influenced by many factors including maternal microbiome, mode of birth, breastfeeding, older siblings, pet exposure,

vaccination, infection and antibiotic exposure in the early life. These early exposures influence the composition of the airway microbiome and provide immunity or increase susceptibility to certain infections (5-7). Studies have shown that there is considerable variation in microbiota between individuals and within individuals at the various sites and over time and this variation extends to geographical diversity which may be influenced by factors such as weather, diet and genetics (8, 9).

Asthma is a chronic inflammatory disease of the airways affecting both adults and children. Asthma is a common condition and has an incidence of 0.67/1000 (95% CI 0.6-0.7) in children aged 0-14 years in the Tuzla canton of the Federation of Bosnia and Herzegovina, with a significantly higher incidence in boys compare to the girls (10). In the UK there are over one million children with asthma (11) and one child is admitted to hospital for an asthma attack every 20 minutes (11). Asthma causation is multifactorial with both genes and environment playing an important role.

One environmental exposure implicated in asthma causation is respiratory tract infection by bacteria and virus, but as with many exposures implicated in asthma causation the relationship between asthma and infection is complex. In some cohorts lower respiratory infection is associated with reduced risk for later asthma (12) but in others the severity and frequency of respiratory tract infections in the early years of life have been linked with increased risk for subsequent development of asthma (5, 6). The 'hygiene hypotheses', described in 1989, proposed that reduced infections (from older siblings) may explain the rise in atopic conditions such as hayfever (13), and over time the underlying mechanism has evolved. A recent expert group have proposed that the "Westernised" lifestyle may mean that the immune system's tolerance of commensal bacteria is replaced by an inflammatory response to bacteria which results in asthma (14).

"Circumstantial evidence" leading to a suspicion that a certain different host bacterial communities ("dysbiosis" is a term which describes microbial imbalance) are important to asthma causation comes from studies which link antenatal maternal antibiotic use, delivery by caesarean section and formula feeding with increased risk for asthma (15-17); these associations support the paradigm that the maternal bacterial community is important to the development of asthma by influencing her offspring's own bacterial community. These associations (15-17) do not prove causation and may arise due to confounding, reverse causation and publication bias. The positive association between early antibiotics and later asthma may also be confounded since "asthmatic" episodes of shortness of breath, cough and wheeze may be diagnosed as "infection" in young children and treated with antibiotic. In addition to the confounding, reverse causation and publication bias, much of the literature linking "early bacterial milieu" to asthma is based on surrogates such as self-reported and retrospectively reported breast feeding. Whilst it is highly plausible that the characteristics of the bacteria in the upper and lower airways are important determinants of asthma, what is required is an objective index of the characteristics of those bacterial communities, e.g. species, burden or diversity

Our aims are to identify and review the literature describing the airway microbiome in children and to explore evidence that links the microbiome to asthma outcomes. The literature search was carried out in August 2019 within the human literature using the keywords microbiome, microbiota, asthma, allergy, pediatric asthma, bacteria, airways in OVID MEDLINE. Further studies were sought from the reference lists of the preliminary search results.

Mechanisms for Microbiome to Cause Asthma

There are several proposed mechanisms which explain the presence of a different microbiome in children with asthma compared to children without asthma (18). These mechanisms are not exclusive:

1. Colonisation with an "abnormal" airway microbiome may cause asthma. Dysbiosis is defined as an imbalance or disruption of the microbial diversity and the presence of a "dysbiotic" com-

munity on the airways may interact with local cells (including epithelial and smooth muscle cells) and cause asthma. The dysbiosis may be in the upper or lower respiratory tract (or both). This model would explain how asthma runs in families (assuming each generation is inoculated with dysbiotic microbiome from their parents) and how asthma may have an onset at any age (assuming dysbiosis can develop at any age). See figure one (scenario A).

2. A primary abnormality of the airway epithelium causes asthma and also provides an environment which leads to dysbiosis (19). This putative airway epithelial cell dysfunction leads to a break in the epithelial barrier which introduces inhaled allergens to the immune system and leading to immune sensitisation (20). In this paradigm the epithelial abnormality would explain the association between asthma and allergy, and here the microbiome present is a result of abnormal airways in asthmatic patients and not a cause of asthma. See figure one (scenario B).
3. Epithelial abnormalities (with associated dysbiosis) in the skin or bowel allow immune sensitisation which in some individuals causes asthma. This paradigm requires an intrinsic pulmonary risk factor to explain why the majority of people who are atopic do not have asthma. See figure one (scenario C).

Differences between Upper and Lower Airway Microbiome

A key question which has not been resolved is whether the upper or lower airway microbiome is more relevant to asthma causation. Since the upper airway (defined as proximal to the vocal cords) is more easily accessed, there are more studies de-

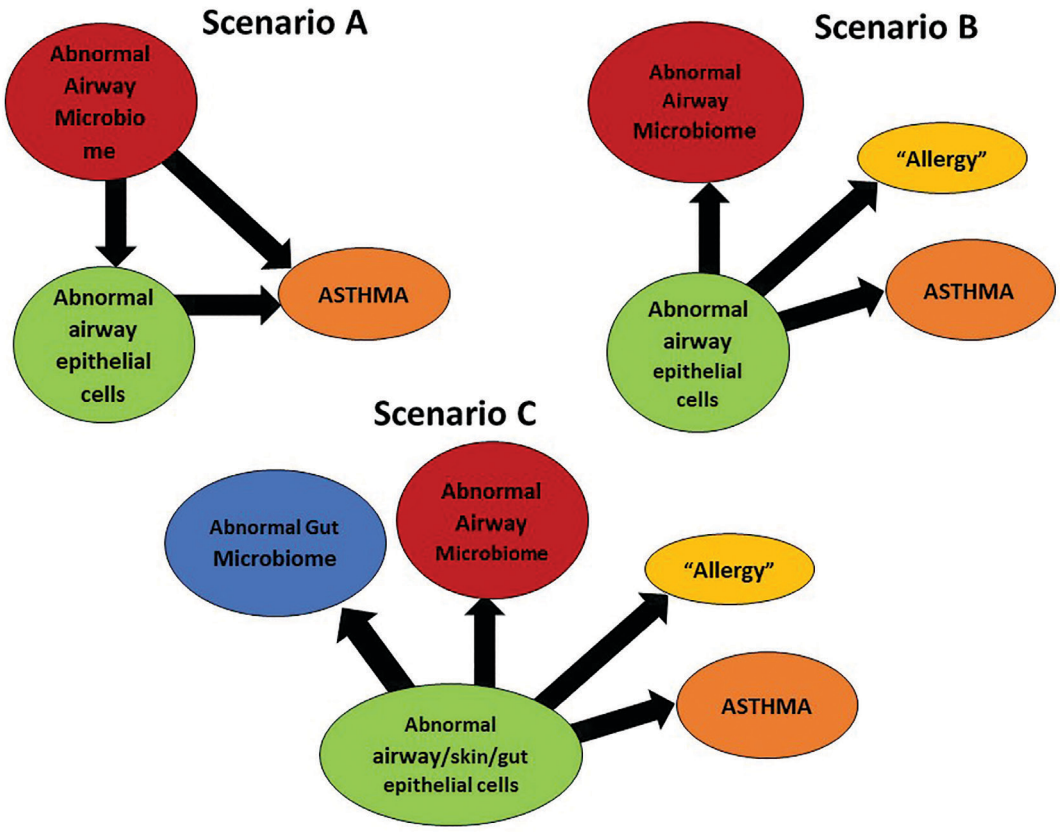

Figure 1. Schematic Diagram Showing Three Different Mechanisms Linking the Airway Microbiome to Childhood Asthma.

scribing associations between the upper respiratory microbiome and asthma outcomes than the lower respiratory microbiome. There are several studies which have compared the microbiome in both upper and lower airways in children with and without respiratory conditions and these demonstrate that the upper airway microbiome is not a reliable index of the lower airway microbiome (3, 21-26). Collectively these studies (3, 21-26) find the greatest difference is between nasal and bronchial microbiome with oro-pharyngeal being intermediate. One group of researchers have suggested using a combination of both nasal and oral microbiota may give a better representation of the lower airway microbiota which could be useful to assess the lower airway without using invasive techniques (21).

These studies make the question whether the upper or lower airway microbiome (at a single time point or over time) is more relevant to asthma causation even more intriguing, since if there are such marked differences between the upper and lower airway microbiome then only one or the other (or neither) is important. The upper airway is the initial point of interaction between inhaled environmental exposures so asthma may develop due to initial interactions in the upper airway, but asthma is a disease of the lower airways and it seems reasonable that mechanisms in the lower airway are more important to asthma causation. In addition, the microbiome not only varies between sites within the human body but also shows variation over time (22). At this point in time we simply do not know the answer to this question and the unified airway concept would argue that the upper and lower airway are essentially the same. What we do know is that there are associations between the upper and lower respiratory microbiome and respiratory outcomes in children. Also there is evidence that the microbiome of the intestine and possibly the skin may be related to asthma or, in the case of the latter, allergy.

Association between the Upper Airway Microbiome and Asthma

Samples from the nose, the nasopharynx and the hypopharynx in early life have been linked to childhood asthma in at least three longitudinal studies. In a prospective cohort study (COPSAC) from Denmark, acute wheezy episodes were significantly associated with presence of bacteria (*Streptococcus, Haemophilus and Moraxella*) in the hypopharyngeal aspirates and this was independent of the significant association with viral infections (23). A follow-up study in the same Danish cohort revealed that children who developed asthma at school age (seven years) had shown an abnormal immune response to these bacteria at 6 months of age with significantly increased levels of IL-5 and IL-10. The authors concluded that inability to clear the pathogenic bacteria from airways in early life can lead to chronic airway inflammation and therefore susceptibility to asthma (24). A similar relationship between the nasopharyngeal microbiome in the first year of life and subsequent development of asthma was observed in an Australian cohort study (25). Table one presents the bacterial phyla associated with later asthma. In a longitudinal analysis of data from a Finnish cohort study (STEPS) there were associations between some species of bacteria (e.g. Moraxella) with an increased risk of respiratory infections later in childhood (26). When the same cohort was followed up, frequency of both upper and lower respiratory tract infections was associated with an increased risk of developing asthma at seven years of age (27). Although there was no attempt to link early life microbiome to later asthma, these two studies suggest that such a link may exist.

In a nested case-control study amongst a cohort of children followed from birth to 18 months of age the authors (28) observed distinct nasal microbiota amongst the cases (rhinitis with and without wheeze) compared to healthy controls. Nasal swabs were gathered from as early as three weeks to three months of age. Authors concluded that nasal microbiome is associated with development of early onset rhinitis and wheeze in infants.

In a cross-sectional case-control study from Korea researchers showed a higher proportion of Firmicutes in upper airway samples from a group with asthma compared to controls and also children with asthma in remission. They also observed

Table 1. Bacterial Phyla Identified in the Upper and Lower Airways which Have Been Linked with Asthma (3, 23, 24, 26, 27, 29-32)

Bacterial phyla in the upper airways associated with childhood asthma	Bacterial phyla in the lower airways associated with childhood asthma
Firmicutes	*Firmicutes*
Actinobacteria	*Actinobacteria*
Proteobacteria	*Proteobacteria*
Bacteroidetes	*Bacteroidetes*
Fusobacteria	*Fusobacteria*
	Tenericutes

higher proportions of *Staphylococcus, Streptococcus, Dolosogranulum* and *Corynebacterium* in the asthma group (31).

Although the relation between bacteria and asthma causation is already complex, a further dimension of complexity may be added by the possibility that viruses may interact by causing reduced bacterial diversity and thereby modify the microbiome-host relationship (33). This interaction between virus-bacteria-microbiome can also play in the other direction a symbiotic relationship, whereby host respiratory bacteria can facilitate binding of Human rhinovirus (HRV) and respiratory syncytial virus (RSV) to the epithelial cells facilitating an inflammatory response, increasing the number of bacterial cell receptors, suppressing the immune system and/ or impacting on the commensal bacteria (34). This was shown in a small longitudinal cohort study of thirty-two infants in Bern, Switzerland (35). Their results showed that symptomatic HRV infections are linked with short term change in bacterial density and diversity and more frequent symptomatic infections have a long-term impact on diversity of the microbiota at the end of first year of life.

Associations between the Lower Airway Microbiome and Asthma

Very few studies have examined the association between lower airway microbiota and asthma. Hilty et al. (3) collected lower airway samples from adults and children with and without asthma and demonstrated how the upper and lower airways have distinct microbiomes and that children with asthma are more likely to have a microbiome rich in members of the Firmicutes phyla such as *Haemophilus* (3). An et al. compared the microbiota in a small sample of children with and without asthma (N=20) and showed an abundance of Proteobacteria amongst those with asthma (N=7) whereas Fusobacteria was the dominant phyla in those without (29). Table one lists the bacterial phyla in the lower respiratory tract which have been associated with asthma.

In another study when researchers analysed both bacterial and fungal populations in samples of the bronchoalveolar lavage, a significant difference was observed in the abundance of bacterial and fungal genera amongst children with and without severe asthma (30). Fungi are also thought to interact with bacteria in the respiratory tract. Pathogenic bacteria such as *P. aeruginosa* and *S. aureus* have been isolated from lungs in Cystic Fibrosis patients along with fungal species of *A. fumigatus* and *C. albicans* (36). The role of the fungi in the airway microbiome and asthma is currently unknown.

Association between the Gastrointestinal Microbiota and Asthma

There is a close relationship between the gastrointestinal (GI) and respiratory tracts, gastro-oesophageal reflux may expose the respiratory tract to upper GI bacteria and fecal-oral transmission may expose the upper airways to lower GI bacteria. The relationship between the gut microbiota

(analysed using fecal samples) and its association with asthma and atopy has been studied in longitudinal cohort studies (37-39). Arrieta et al. have shown that dysbiosis of the gut microbiota (fungal and bacterial) in the first 100 days of life is a risk factor for the development of atopic wheeze in children (37). Lack of abundance of certain species such as *Bifidobacterium, Akkermansia, Faecalibacterium, Roseburia* and a higher abundance of the bacterium *Veillonella* and the fungi *Candida* and *Rhodotorula* was linked with an increased risk of developing asthma in some of these studies. Results from a Danish cohort study revealed that lack of maturation of the gut microbiome in the first year of life contributed to increased risk of developing asthma at age five (39). In the same study maternal asthma although did not affect the microbial colonies of children it did act as an effect modifier increasing their risk of developing asthma and this the researchers associated with inborn immune deviation.

Conclusion

We are currently at the start of a line of enquiry into the role of the microbiome in childhood asthma. When the paediatric and adult data are considered, it is almost beyond reasonable doubt that the microbiome in the upper and lower airway is different in people with asthma compared to their peers who do not have asthma. What remains to be determined is whether a dysbiosis is the cause or the result of asthma. Longitudinal studies are required to determine the true nature of the relationship between the microbiome and asthma. These studies also need to consider:

- The role of viral infection and fungal colonisation in the airways,
- Using a robust objective index of airway function as an outcome alongside the rather subjective "asthma",
- Confounders including genetic factors and antibiotic use and
- There are likely to be different asthma endotypes, only one/some of which are related to the microbiome.

The COPSAC data (24) provide encouragement to this exciting line of research. Almost 40 years ago it was suggested that a chronic eosinophilic inflammatory condition in a derivative of the embryonic foregut (i.e. peptic ulcer disease in the stomach) was caused by infection. This suggestion was thought highly unlikely until *H pylori* was isolated in the stomach mucosa. Is it possible that an infection of a currently "unknown" bacteria might cause a chronic eosinophilic inflammatory condition in another derivative of the embryonic foregut? Let's see.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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Influence of Innate Immunity on Immune Tolerance

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Abstract
This review mainly focuses on the mechanisms of peripheral immune tolerance within the perspectives of innate immunity. Healthy immune response requires balanced interaction of the highly specialized elements of immunity within a harmony. Innate immunity supported by microbial pattern recognition receptors, physical anatomical barriers and soluble effectors stands as the first line of defense against non-self-antigens. Innate receptors recognize major classes of pathogens and trigger immediate immune/inflammatory responses. The decisive action has been the key issue in skewing of immune reactivity to a pathogen or to tolerate self- and non-self-antigens. Non-responsiveness to self- or to harmless foreign antigens with means of multiple mechanisms is known as immune tolerance; a non-inflammatory, non-proliferative and suppressive response linked to suppressor molecules as CTLA-4 and cytokines like IL-10, TGF-β and IL-35, and also to non-inflammatory blocking antibody isotypes as IgG_4. Regulatory cells ascertain both induction and maintenance of peripheral tolerance. Allergic diseases, autoimmunity and transplant rejection are the best illustrations of immune tolerance loss. Adaptive immunity responsible for both establishment and maintenance of a long-lasting immune responsiveness is mainly fine-tuned by actions of innate immunity. Better understanding of the relationship between innate immunity and immune tolerance is a prerequisite both for better understanding of pathogenesis of tolerance-related diseases and also for development of novel therapeutic options. **Conclusion.** Recent evidences point the important roles of innate immunity for establishment of immune tolerance with decisive role in central mechanisms. In a peremptory way, a '*balanced tolerance*' is essential for the survival.

Key Words: Antigen-Presenting Cells ▪ Cytokines ▪ Central Immune Tolerance ▪ Innate Immunity ▪ Peripheral Immune Tolerance.

Introduction

Immunity is comprised of specific and non-specific responses generated by several tissues, cells and their products, which functions elegantly within the customized networks of interactions. This system has to distinguish pathogens from external innocuous antigens and self-antigens to determine the intensity and the class of immune responses to be generated. Non-responsiveness, either to self or to non-self antigens, is defined as immune tolerance that is formed and maintained by combined means of the central and peripheral immune tolerance mechanisms. Dysregulation or loss of immune tolerance may lead to the development of allergic disorders such as asthma, allergic rhinitis (AR), atopic dermatitis and more, besides autoimmunity, recurrent abortions and transplant rejections (1).

This review mainly focuses on the mechanisms of formation and maintenance of peripheral immune tolerance within the perspectives of innate immunity.

Immune System and Immune Tolerance

Both arms of innate and adaptive immune systems work in a harmony to generate healthy im-

mune responses. Epithelial barriers, dendritic cells (DCs) and phagocytes, natural killer (NK) cells, innate lymphoid cells (ILCs) and elements of the complement system constitute the major components of innate immune system, while T and B lymphocyte functions, generated antibodies, in addition to responses of effector cells such as mast cells, basophils and eosinophils form the adaptive arm of the immune system. It has to be noted that the adaptive immune responses require both recognition of specific antigens and innate immune signals for custom-tailoring of immunity to a particular antigen.

The immune system is capable of tolerating both self- and non-self-antigens by means of the central and peripheral immune tolerance mechanisms. This is crucial for immune homeostasis. A well-balanced tolerance facilitates the survival and stabilizes the organism, while excessive tolerance may lead to inadequate defense against the invading pathogens and inadequacy to limit the development of chronic infections and cancer. On the other hand, loss of tolerance to external antigens may induce hypersensitivity reactions as seen in allergic disorders, while loss of tolerance to self-antigens may lead to the development of autoimmune diseases (2).

Pregnancy stand as the best model to exemplify how optimum and perfect the immune tolerance has to be. Maternal immune system has to tolerate fetal antigens until delivery in order to avoid fetus rejection (3). Accordingly, it will be appropriate to interpret these functions of the immune system in a way that 'a healthy immune response' can be defined as generating optimum and sufficient responses against pathogens and cancer cells, while tolerating self-tissues, harmless environmental antigens and the commensal microorganisms.

Establishment of immune tolerance is determined by two major mechanisms: the central and the peripheral immune tolerance mechanisms. During development, in the case of recognition of self-antigens by progenitors of either B cells in bone marrow or T cells in thymus, the silencing or deletion of these cells form central tolerance. Although a great percentage of developing cells are deleted in central lymphoid organs, a small number of T cells can still escape from thymic deletion and reach to periphery (4, 5). This is regulated by means of peripheral tolerance through apoptosis, anergy and T regulatory (Treg) cell action in the secondary lymphoid organs (6). Peripheral tolerance mechanisms are also responsible for healthy immune responses to environmental antigens, including allergens (6). It has been depicted that the ratio of the effector T helper (Th)2 cells to the Treg cells for the same particular antigen form the basis in determining allergic or healthy response in an individual (7). Other immune cell subsets with regulatory capabilities also contribute to tolerance, including NK cells, ILCs and B regulatory (Breg) cells (7-9). The ultimate response to a certain antigen could be defined by interactions between the environment and the immune system, where the innate immunity indispensably contributes to the determination of the response type to a particular antigen (10). Better understanding of innate immunity is essential for establishment of the links between innate and adaptive immune systems and their interactions with the environmental stimuli.

Innate Immunity Defining the Fate: Immune Tolerance or Immune Response

The decisive factors on the crossroads of responsiveness or unresponsiveness are being deeply investigated nowadays. Studies have revealed indispensable roles of Treg cells in the establishment and the maintenance of peripheral tolerance. Treg cells belong to a specific sub-group of $CD4^+$ T cells of the adaptive immunity. They express the forkhead box P3 (FoxP3) transcription factor, and have suppressive capacities by production of cytokines such as interleukin (IL)-10, IL-35 and transforming growth factor (TGF)-β, and by surface expression of suppressor molecules as cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) (11-13). Treg cells contribute to allergen-specific tolerance and can be induced as a consequence of allergen-specific immunotherapy (AIT) (14, 15). In parallel with the mechanisms of allergen tolerance, a group of self-peptide specific $CD4^+$ T

cells can be converted to Treg cells under certain conditions and contributes to regulation of autoimmune responses in periphery (16). At first look, adaptive immune system which is responsible for the establishment and maintenance of a long-lasting antigen-specific tolerance is orientated with the early actions of innate immunity. In addition, early innate immunity is important to define the intensity and class of immune tolerance. For better understanding of the role of innate immunity in immune tolerance development, contribution of cellular players of innate immunity and their cytokines, as well as other factors will be discussed throughout this review.

Innate Cytokines Acting on Tolerance

Almost all immune cells like DCs, $CD4^+$ and $CD8^+$ T cells, B cells, NK cells, and ILCs have functional subsets, all of which are categorized according to their distinct surface receptors and cytokine secretion patterns. Cytokines such as IL-1β, IL-6 and tumor necrosis factor (TNF)-α exert proinflammatory properties, while IL-10, IL-27, IL-35 and TGF-β are known for their suppressive effects (17).

Innate Immune Functions of Interleukin-10 is crucial in establishment and maintenance of immune tolerance. IL-10 is the most widely investigated and the best-known suppressive cytokine to date. IL-10 is mainly produced by monocytes, Treg cells, Breg cells, a small fraction of NK cells, macrophages, as well as by DCs and mast cells (17). IL-10 has a direct innate effect, which suppresses antigen presentation and development of adaptive immunity. IL-10 limits production of proinflammatory cytokines such as TNF-α, IL-6 and IL-8, inhibits conversion of DCs from monocytes and restricts presentation capacity of antigen presenting cell (APC) by down-regulation of surface molecules as major histocompatibility complex (MHC)-II and CD86. Moreover, IL-10 controls T cell responses by restricting IL-2, IFN-γ and granulocyte-macrophage colony stimulating factor (GM-CSF) productions from T-cells and limits T cell proliferation (18, 19). IL-10 induces expression of a number of molecules with tolerogenic properties in human monocytes, macrophages and DCs, as well. Innate inflammatory cytokines like IL-1 contributes in tissue recruitment of leukocytes in response to an injury, by increasing the expression levels of adhesion molecules; intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on endothelial cells. In contrast, IL-10 down-regulates the expression of both of these adhesion molecules, which results in decreased migration of proinflammatory cells at the site of tissue injury and in turn limits inflammation (20).

Studies highlighted the importance of IL-10 in maintenance of tolerance. DC-derived IL-10 production observed in respiratory tract of healthy individuals was found to be diminished in patients with AR and asthma (1). In the gastrointestinal tract, IL-10 is produced both by intestinal macrophages and by Treg cells and contributes to mucosal homeostasis. Loss of IL-10 receptor in APCs was related to epithelial damage in the intestines (21). Generation of peripheral tolerance in allergic individuals occurs by induction of Treg cells either by natural high dose allergen exposure, as seen in beekeepers (22) and in food allergic children (23), or induced by allergen-specific immunotherapy (24). Induced Treg cells produce high amounts of IL-10 and bring forth the above mentioned effects (25).

There are several instances, particularly in infections and tumors in which IL-10 detrimentally contributes to immunity. During leishmania infection, partial blockade of IL-10 in localized cutaneous leishmaniasis was found to be beneficial for patients (26). In addition, IL-10 contributes to impaired bacterial clearance in *Mycobacterium tuberculosis* infection (27). As revealed in a meta-analysis, increased serum levels of IL-10 in cancer patients with solid tumors or hematological malignancies was found to be predictive for a worse outcome (28). Taken together, this important immune regulatory cytokine has two opposite facets, while maintaining tolerance, the organism could become prone to both infections and cancer. Therefore, the general immune suppressor activity of IL-10 becomes detrimental in immune responses to infectious agents and tumors.

Transforming Growth Factor-Beta is known for its pleiotropic properties. It is produced by assortment of cells including lymphocytes, macrophages, eosinophils, epithelial cells and fibroblasts, and it influences almost all cell types due to widely expressed TGF-β receptors (17). TGF-β is among the most important cytokines produced by Treg cells, with well-known suppressive effects (29). TGF-β has anti-inflammatory effects on innate cells like NK cells, monocytes and macrophages (30). In addition, TGF-β influences adaptive immunity by suppression of Th1 and Th2 type $CD4^+$ T cells. It contributes to differentiation of Th17 and Th9 cell subsets from naïve T cells and plays a role in the induction of conventional Treg cells and also by particularly upregulating the expression of their signature transcription factor; FoxP3 (31). In airway diseases like asthma, chronic rhinosinusitis and allergic rhinitis, TGF-β contributes to both inhibition of T-cell responses and inflammation, and also to tissue repair and remodeling processes (32). In all of these diseases, a chronic mucosal wound that needs to be healed is a part of the pathogenesis and TGF-β is the key cytokine that comes to the scene for this activity. Immune suppression affects various cells around the wound and induces fibroblast proliferation at the same time. This effect cannot lead to full recovery, but causes a continuous inadequate healing process that is recognized as remodeling. TGF-β is well known with its contribution to tissue remodeling in allergic diseases, especially in allergic asthma, with hallmarks such as thickening of basal membrane, increased smooth muscle mass in bronchi, increased mucus secretion due to goblet cell hyperplasia and infiltration of immune cells to the inflamed area, all of which could contribute to a worse disease prognosis (33). TGF-β2 is overexpressed in fibroblasts of severe asthmatics, which leads to proliferation of epithelial cells (34). Though well-known as an immune regulatory cytokine, TGF-β has pleiotropic effects, therefore this cytokine could be responsible for irreversible changes in certain disease conditions. More studies are required to elucidate the contribution of TGF-β both in immune regulation and in remodeling and also in the balance between these key events.

Interleukin 27 belongs to IL-12 family of cytokines with both pro- and anti-inflammatory properties, which are attributed to plasticity and cross-talk of cytokine subunits in addition to shared utilization of receptors with other cytokines (35, 36). IL-27 is expressed by myeloid cells like monocytes, macrophages and DCs, while its receptors are expressed widely on leukocytes including NK cells and lymphocytes (37). IL-27 plays important roles in Th1 differentiation via controlling IL-12 responsiveness (38). Intracellular glutathione redox status in monocyte-derived DCs regulates production of IL-27, which contributes to Th-1-polarizing effects of DCs (39). Anti-inflammatory functions of IL-27 can be mediated by induction of IL-10 production in $FoxP3^-$ $CD4^+CD25^+$ T regulatory type-1 (Tr1) cells (40, 41), and IL-10 production of $CD4^+$ T cells under Th1 and Th2 polarizing settings can be augmented by IL-27 (41). On the other hand, IL-27 suppresses the development of $FoxP3^+$ inducible regulatory T cells (iTregs) and Th17 cells via differential effects on STAT1 transcription factor (42). Briefly, IL-27 regulates diverse Th cell subsets, limits excessive activation of T cells and may have potential applications both in AIT and controlling of autoimmune diseases (43).

Interleukin-35 is a recently discovered cytokine of IL-12 family with immune regulatory roles. It is produced by Breg, Treg, epithelial and vascular endothelial cells as well as by immature DCs (44-47). A minor Treg cell group, with capability of secreting IL-35, is dependent on the expression of FoxP3 (48). Treg-derived IL-35 limits Th1, Th2 and Th17 responses and controls inflammation (49, 50). IL-35 has been proposed to act on limitation of an already established inflammation and thus may exert regulatory effects in autoimmune diseases like rheumatoid arthritis and systemic lupus erythematosus. Increased levels of IL-35 in autoimmune disorders may indicate a compensatory attempt to down-regulate the inflammation (51). Contribution of IL-35 in the pathogenesis of allergic diseases is questioned. Murine models of asthma have pointed out suppressive roles of IL-

35 on airway hyper-reactivity and inflammation (52). A recent study in patients with grass pollen allergy asserted IL-35 as a novel immune regulator induced following grass pollen sub-lingual immunotherapy (53). On the other hand, this regulatory cytokines' potential detrimental effects within the frame of tumor immunology have been revealed. IL-10 and IL-35 produced by Treg cells in tumor micro environment cooperatively regulate anti-tumor immunity by promotion of T cell exhaustion, could underlie a tumor immune evasive strategy and form a potential immune resistance mechanism to tumor immunotherapy (54). Taken together, future studies are required for determination of properties of IL-35 to be utilized in therapeutic interventions.

Interleukin-37 is a member of IL-1 family of cytokines expressed in NK cells, monocytes, stimulated B cells and epithelial cells. Low physiological expression of IL-37 is upregulated by inflammation and anti-inflammatory roles for IL-37 have been proposed. Accordingly, IL-37 inhibits both innate and adaptive immune responses, decreases production of pro-inflammatory cytokines from DCs and macrophages, and contributes to a number of chronic autoimmune and inflammatory disorders, cardiac diseases, as well as cancer (55). Diminished production of IL-37 in human peripheral blood mononuclear cells of allergic asthmatics in comparison to their healthy counterparts have been reported. The same study also revealed remission of airway inflammation, cytokine production and mucus secretion in a mouse model of asthma (56). Recently, a study investigating the contribution of IL-37 to allergic inflammation has revealed diminished airway hyper-reactivity and pulmonary eosinophilia in a mouse model of house dust mite-induced asthma, in response to IL-37 (57).

Innate Immune Cells and Immune Tolerance

Antigen-presenting cells including DCs, macrophages and B cells contribute to the initiation of immune responses and are therefore important sentinels of immunity. DCs with superior antigen-presenting capacity engulf, phagocyte and process antigens into peptide fragments and then present on their surfaces to different subsets of T cells via MHC molecules. During antigen presentation, co-stimulatory signals to T cells are provided by molecules expressed as CD40, CD80 and CD86, and they also provide polarizing cytokines to T cells, all of which act together to define the fate of the T cell responses to be either on effector or regulatory side (58). Therefore, it may be hypothesized that the development of tolerance versus reactivity may be initially driven by DCs that are capable of forming links between innate and adaptive immunity with vital roles in the orchestration of immune responses (59). Maturation status of DCs together with antigen presentation levels define the outcome either to be tolerogenic or immunogenic (Figure 1). DCs at steady state conditions express low-levels of maturation markers such as MHC-II, CD40, CD80 and CD86, and inflammatory cytokines, which can be termed as immature and are known to promote induction of tolerance. Following activation by antigens, migration to the lymph nodes together with the initiation of DC maturation and up-regulation of these maturation markers are essential for T cell activation. On the other hand, some commensal microorganisms as well as tonic inflammatory signals are not sufficient to induce high-level expression of maturation markers in steady-state DCs. Therefore, these cells do not acquire immunogenic capacities (60). Diminished expression of MHC-II together with down-regulated levels of co-stimulatory molecules on DCs brings out their tolerogenic properties, which in turn leads to T cell suppression, a requisite for establishment of tolerance (60).

The fate of immature dendritic cells (DCs) following antigen uptake is determined by the innate immune response related factors present in the micromilieu. A DC uptaking the same antigen has a capacity to act in opposite ways according to the surrounding conditions. Under inflammatory conditions, the immature DC migrating from site of injury/inflammation to the lymph nodes will be initiated and during DC maturation, expression levels of MHC-II molecules together with the co-stimulatory molecules: CD80 and CD86 and in-

flammatory cytokines; IL-1, IL-12 and IFN-γ will be upregulated. The net results are increased antigen presenting cell (APC) capacity, which is sufficient for initiation of adaptive T-cell responses, and production of cytokines relevant with pathogen type that will drive induction of different Th subsets such as Th1, Th2 and Th17. The aberrant activation may have pathogenic consequences as seen in autoimmunity, allergic diseases and graft rejection. On the other hand, during antigen uptake, if there is no inflammation, if cytokines with known suppressive effects such as IL-10 and TGF-β are present, or if TLR2-, TLR7- or TLR9-triggering PAMPs are present in the micro milieu, then immature DCs will be converted to tolerogenic DCs characterized with low expression levels of CD80 and CD86 together with low MHC-II. These cells can produce TGF-β and IL-10, and they can express suppressive molecules such as immunoglobulin-like transcript (ILT)-2, ILT3 and ILT4. Vitamin D3 as well as retinoic acid and indoleamine 2,3-dioxygenase (IDO) contribute to the induction of tolerogenic DCs. These DCs have capacity to induce T regulatory cells, which have suppressive effects to prevent pathologies. (Blue arrows indicate tolerogenic conditions, while red arrows indicate inflammatory conditions.)

A great number of experimental studies have investigated the contribution of DCs to the establishment and maintenance of immune tolerance. A mouse model revealed that both production of IL-10 and down-regulation of MHC-II expression were important factors that contribute to the tolerance induced by IL-10-differentiated DCs (61). IL-10, produced by DCs or other cells, inhibits gene transcription of pro-inflammatory cytokines together with down-regulation of MHC-II and co-stimulatory molecule expressions in activated DCs. This results with restricted presentation of antigens to T cells and ends up with decreased T cell activation (62). Together with these well-known facts, a recent experimental study revealed upregulation of FoxP3 in mouse DCs under certain conditions, which could possibly have a potential application for future therapeutic approaches (63).

Inhibition of monocyte-conversion to inflammatory DCs by IL-35 was shown to ameliorate ovalbumin-induced allergic inflammation in mouse

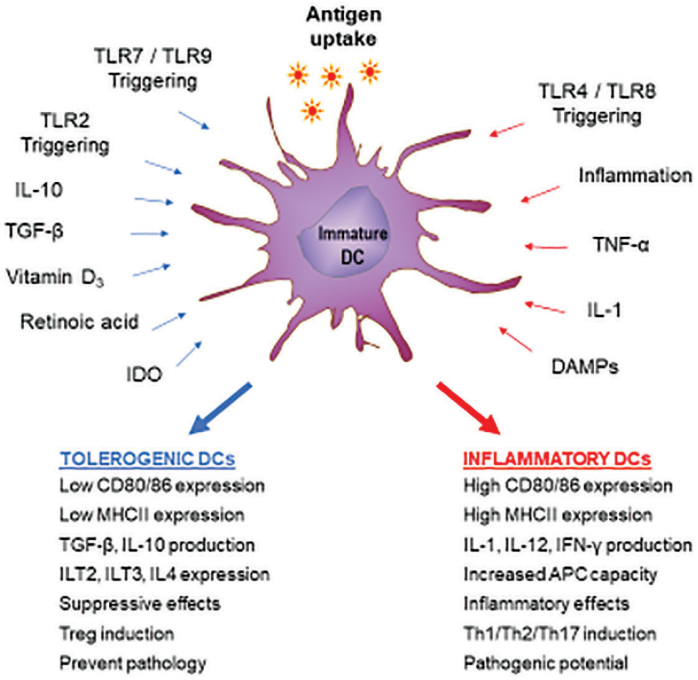

Figure 1. Influence of Innate Immune Factors on Dendritic Cell Responses.

models, leading to T-cell tolerance to ovalbumin antigen (64). A number of studies in humans has also tried to establish a link between DCs and immune tolerance. Studies investigating the oral cavity, the place where diverse environmental antigens are encountered such as commensal microbes, dietary antigens and allergens, have revealed the presence of several subsets of tolerogenic DCs with the capacity to induce immune regulatory responses in humans (65).

By oral administration of innocuous antigens such as food allergens, immune microenvironment is modulated, tolerogenic immune modulators are increased, all of which can inhibit inflammatory responses and lead to induction of tolerance (66, 67). Cross-talk between the host and microbiota has great importance in designation of immune responses. Important propensities of some bacteria and also their microbial particles for impelling DCs attitude towards a tolerogenic response have been reported. The tolerization of inflammatory epidermal DCs as well as skin Langerhans cells occur in response to toll-like receptor (TLR)2-mediated recognition of *Staphylococcus aureus* in atopic dermatitis skin (68). Lipopeptides of gram-negative bacteria are known to regulate immune responses. A potent analogue of synthetic lipopeptides; LP40 incorporates TLR2-dependent mechanisms in DCs that enhances production of IL-10 and IFN-γ, limits naïve T cell differentiation and indirectly limits production of IL-4 together with limitation of IgE- and promotion of IgG4-antibody isotypes. All these mentioned effects are potent limitation factors for Th2-type allergic inflammation (69).

Plasmacytoid DCs are known to produce anti-viral type-1 interferons upon TLR7 and TLR9 stimulation, and are revealed to contribute to the establishment of peripheral tolerance during AIT (70). Tonsils are strategically located lymphoid organs in the entrance of both respiratory and alimentary tracts, where first contact of the immune system with the food and respiratory antigens take place. Both pDCs and mDCs are present within the tonsils, with the dominance of pDCs that are co-localized with FoxP3$^+$ Treg cells. Diminished numbers of pDCs observed in tonsil samples of atopic individuals revealed significance of this APC subset in induction of immune tolerance (71). In contrast, mDCs and stimulations that can activate them are related with loss of allergen-specific T cell tolerance both in blood and tonsil samples of healthy, non-allergic individuals (72). Studies demonstrating the contribution of pDCs in prevention of atherosclerosis (73) and allograft rejection (74) also support the tolerogenic roles of pDCs by inducing Treg cells.

Current research has exposed functionally abnormal DCs in allergic patients, which may be modulated towards a tolerant state by interventions like AIT, corticosteroids, some DC-related cytokines as thymic stromal lymphopoietin (TSLP) (75). Studies have also intended to develop utilization of DCs in induction of tolerance. As an example, a mouse model utilizing allergoids coupled with mannan to be uptaken by oral mDCs via sublingual route has revealed the induction of FoxP3$^+$ Treg cells (76). Protocols for induction of DCs with tolerogenic capacities (tol-DCs) have been defined. Following differentiation of DC precursors with mediators like IL-10, TGF-β or dexamethasone, these cells acquire tolerogenic properties (77), which is promising for promotion of antigen-specific immune tolerance. DC-10, an inducible subset of tol-DCs, secretes sensible amounts of IL-10, expresses tolerogenic molecules including ILT2, ILT3, ILT4 and HLA-G and promotes anergy of T cells together with induction of Tr1 cells via IL-10 dependent ILT4/HLA-G pathway (62, 78). Studies have revealed important contribution of DC-10 in pregnancy and linked low frequencies of DC-10 in decidua of women with early miscarriage, which is of noteworthy (62). Understanding the DC biology is utmost important both for better illumination of their roles in precise-trimming of immune responses and possible utilization of DCs in future therapeutic interventions.

Contribution of Programmed Death Receptor in Immune Regulation Co-stimulatory signaling through B7 family members is a prerequisite both for activation and inhibition of T cell responses.

Programmed death-1 receptor (PD-1) was initially identified in T cells undergoing apoptosis (79) and is known to contribute to the establishment and maintenance of immunological tolerance. PD-1 has two ligands; PD-L1 (B7-H1) and PD-L2 (B7-DC), and triggering of PD-1 with these ligands contributes to negative regulation of T lymphocyte activation, proliferation, survival and cytokine production (80), and also induction of Treg cells (81). PD-1 is expressed in a number of cell types including T cells, B cells, monocytes, mesenchymal stem cells and Treg cells. Although having similar roles and binding to the same receptor, PD-L1 and PD-L2 have different expression patterns. PD-L1 expression is observed on macrophages, DCs and B cells, while expression of PD-L2 is limited to macrophages and DCs following activation (82-84). PD-1 has protective roles against autoimmune disorders through induction of T cell apoptosis and promotion of Treg cells while PD-1 blockade was reported to have promising outcomes in tumor immunotherapy (81). A number of studies have investigated the contribution of PD-L1 and PD-L2 in mechanisms of allergic disorders. PD-L2 and PD-L1 were claimed to have opposing effects in airway inflammation. PD-L2 was found to contribute to the regulation and suppression, while PD-L1 was claimed to be decisive for development of airway hyperreactivity in a mouse model of asthma (85). In addition, increased expression of PD-1 observed in nasal tissue samples of patients with chronic rhinosinusitis with nasal polyps was correlated both with disease severity and tissue expression of IL-5 (80). Further studies focused on contribution of these molecules in allergic inflammation may provide a potential for the discovery of new biomarkers potential and also novel therapeutic interventions.

Natural killer cells, a subset of lymphocytes, are best known for their cytotoxic properties against tumors and virus-infected cells. NK cells contribute to immune responses with their cytokine productions. Their activity is tightly regulated by the balance of cell surface inhibitory and activating receptors. Surface marker designation for phenotypic characterization of NK cells is as $CD3^-$ $CD16^+$ and $CD56^+$. Recent studies have revealed two major subsets of NK cells in peripheral blood with respect to their CD16 and CD56 expressions. NK cells with high expression of CD16 and low expression of CD56 ($CD16^+CD56^{dim}$) form the majority of the circulating NK cells, while the remaining NK group is characterized with no expression of CD16 and high expression of CD56 ($CD16^-$ $CD56^+$) (86, 87). These subsets have discrete functional properties; $CD16^+CD56^{dim}$ NK cell subset has cytotoxic properties and a limited potential for cytokine secretion (88). On the other hand, $CD16^-$ $CD56^+$ NK group has been characterized with high cytokine secretion competency, which may play roles both in inflammatory and regulatory properties, in response to various stimuli (9, 89). NK cell groups exist with respect to their cytokine secretion profiles analogous to Th1 and Th2 cells (90), while a subset of NK cells that produce IL-10 *in vitro* is termed as NK regulatory subset that can suppress antigen-specific T cell responses (9). A number of studies proposed roles for NK cell derived IL-10 in inflammatory disorders such as Behcet's disease and multiple sclerosis (91-93). NK cells respond to a variety of cytokines, such as IL-2, IL-15, IL-12 and IL-18 and the effectual microenvironment can modulate the functions of these innate cells. NK cells have the capacity to produce important cytokines such as IFN-γ, TNF, IL-5, IL-13, IL-10, GM-CSF and also chemokines such as CCL3, CCL4, CCL5 and CCL8, all of which propose important potentials for contribution to the regulation of immune responses (94, 95).

Better understanding of how NK cells contribute to the regulation of inflammation has an important value to find solutions for inflammatory diseases and tissue injury. In humans, NK cells were revealed to utilize cytotoxicity towards DCs, $CD4^+$ and $CD8^+$ T cells, which can limit excessive immune responses in viral infections. Suppression of $CD4^+$ T cells by NK cells in turn limits B cell-mediated humoral immunity against viruses, all of which are important for the establishment of a critical balance between efficient immunity versus excessive and tissue damaging inflammation (96-98). In a mouse model, where excessive

inflammation was triggered by lymphocytic choriomeningitis virus infection, complete depletion of NK cells or presence of NK cells, which lacks perforin-mediated cytotoxicity worsened the disease progression. $CD8^+$ T cell cytotoxicity was revealed to be controlled by NK cells in this model (99). For comparison, in asthma, a decrease in total NK cell counts, increased ratio of the cytotoxic NK cell subset with diminished cytotoxic capacity and an inverse correlation with lung function were observed (100). In another study, NK cells were found to contribute to eosinophil apoptosis in non-severe asthma and diminished NK activity due to the lack of a pro-resolving mediator; lipoxin A4 was observed in severe asthma (101). Taken together, understanding the roles of NK cells, the important sentinels of innate immunity, which contribute to both the aggravation and the regulation of inflammation beside their best-known action of cytotoxicity against the tumor cells and virus-infected cells, will enable us to utilize them as new therapeutic interventions.

Innate lymphoid cells are recently discovered subset of lymphocytes, which lack lineage markers and antigen-specific receptors of T or B cells. ILCs have been revealed to contribute to immune responses by their cytokine secretions, which are similar to their corresponding Th cell subsets as ILC1, ILC2 and ILC3 (similar to Th1, Th2 and Th17, respectively). They serve as a bridge in between innate and adaptive arms of immunity (102, 103). ILCs are oriented by lipid mediators, as well as cytokines produced by stromal, epithelial and myeloid cells and contribute to trimming of immune responses by their cytokine and mediator productions and via cell to cell contacts (104). ILCs contribute to both allergic and non-allergic diseases of inflammatory origin by producing cytokines and other mediators (104). ILC2s exert roles in allergic diseases by augmenting the Th2-type inflammation. Peripheral ILC2s which could contribute to induction of Th2 cells from naïve T cells were revealed to be diminished as a result of subcutaneous allergen immunotherapy in patients with severe seasonal allergic rhinitis (105). In line with this report, a recent study has revealed diminished numbers of peripheral blood ILC2s in response to AIT, in patients with AR (106). Likewise, a recent study has revealed regulation of ILC2 functions in allergic diseases by Tregs and their relevant cytokines, IL-10 and TGF-β (107).

Among ILCs, a specific subset have the capacity to enhance an immunologically tolerant state (108, 109). A recent study has reported a subset of IL-10 producing ILCs with regulatory functions, termed as ILCreg, which are present in the gut and were shown to have a unique gene identity that do not resemble classic ILCs or other Treg cells. TGF-β1 was determined to be produced by ILCregs during innate intestinal inflammation, and autocrine TGF-β1 has been revealed to sustain the expansion and maintenance of ILCregs, all of which propose a possible role for ILCs in maintenance of tolerance by innate immunity (8). Recently, retinoic acid, a vitamin A metabolite was shown to induce IL-10 producing ILCregs from ILC2s. This subset had a profile similar to Tregs by expression of CTLA-4 and CD25 together with diminished production of Th2-type cytokines (110). ILCs attract attention and are better characterized and understood day by day. Better understanding of their contribution to immune responses require extensive studies, however, as this rare subset of innate immune cells contributes to almost all diseases, it is expected that they may have roles beyond the current concepts.

Basophils and mast cells as their tissue counterparts; are members of innate immunity and contribute to Th2 immune responses. Mast cells and basophils are known to be loaded with granules and once triggered by cross linking of IgE bound to their Fcε receptors, they rapidly degranulate, synthesize and release intra-granular mediators including histamine, proteases, cytokines and lipid mediators (2). Reportedly, basophils that produce IL-3 promoted Th2-type airway inflammation in a mouse model (111). Th2 type immune responses are associated with immunity against parasites and when Th2 responses are directed to allergens, allergic reactions are manifested. In addition to their rapid effector responses to environmental stimuli as allergens, mast cells also exert modulatory effects

on immune responses (112). Functional interplay between mast cells and T cells has been proposed (113). Mast cells have regulatory and immune suppressive properties and are involved in the induction of immune tolerance (114). In experimental studies, mast cells were revealed to contribute to peripheral tolerance in response to Treg derived IL-9 (115, 116). On the other hand, bone marrow derived mast cells are known to induce an increase in the levels of $CD4^+CD25^+FoxP3^+$ Treg cells in a TGF-β1 dependent manner (117). Human mast cells produce both IL-10 and TGF-β which may contribute to immune regulation (118, 119). A recent study revealed basophil anergy in a house dust mite allergic population with an observed amelioration of AR. Basophil anergy was proposed to be a promising phenomenon both for being a biomarker and for a possible utilization in therapy, which deserves further investigations (120).

Other Players Acting on Immune Tolerance

Histamine*;* a biogenic amine, is an important mediator of allergic inflammation that induces vasodilatation, increases vascular permeability and contributes to type-1 hypersensitivity reactions. Histamine exerts its physiological functions like differentiation and proliferation of cells and hematopoiesis (121). Histamine has pleiotropic effects attributed to its 4 different receptors (H1R, H2R, H3R, H4R), all of which represent a complex immune regulatory role with discrete effects in relation with the receptor subtypes and their differential expressions (122). Among these receptors, H2R with relatively high expression on Th2 cells, contributes to the induction of Treg cells and induction of peripheral tolerance to allergens. In response to H2R up-regulation, IL-10 production increases and T cell stimulation is down-regulated. H2R has been shown to enhance the suppressive effects of TGF-β on T cells, which supports the role of histamine in H2R-mediated immune regulation (123-125). H2R has also been revealed to have effects on limitation of lung inflammation by regulation of lung invariant NKT (iNKT) cell responses (126). On the other hand, H4R is expressed in several hematopoietic cells and plays essential roles in the histamine-induced activation of DCs, monocytes, T cells, mast cells and eosinophils. H4R exerts its functions in both autocrine and paracrine manners. Triggering of H4R increases the expression of adhesion molecules, shapes and arranges the actin cytoskeleton of eosinophils that in turn lead to increased movement of these cells. H4R also plays roles in mast cells to mobilize calcium and induces chemotaxis without affecting degranulation, enabling the selective recruitment of these cells (127, 128).

Innate Mechanisms of Immune Tolerance-Related Disorders

As mentioned above, immune tolerance is a state of unresponsiveness to immune stimuli that otherwise have the capability to generate immune reactivity. Although suppression of this reactivity is relatively a desired condition when taken from the allergy and autoimmunity points of views, excessive suppression, in other words, superfluous tolerance, may promote emergence of chronic infections and cancer development (Figure 2). It is reasonable that, loss of tolerance may also be a key step in the development of allergic disorders, autoimmunity, host versus graft reactions following transplantations and miscarriages leading to infertility.

A fine balance between reactivity (effector functions) and non-reactivity (tolerance) should be established for a healthy state. Excessive tolerance to antigens could end up with tumor development and/or chronic infections, on the other hand, over reactivity could end up with allergic diseases, auto immunity, rejection of transplanted tissues or organs, and infertility. Cytokines acting on induction and maintenance of tolerance; IL-10, TGF-β, IL-27, IL-35 and IL-37 are secreted by cells that have regulatory capacities. Mast cells, basophils as well as APCs have capacity either to induce or break tolerance, which is determined by some specific stimulations. TLR7 and TLR9 support a tolerant state while triggering of TLR4 and TLR8 induce secretion of proinflammatory

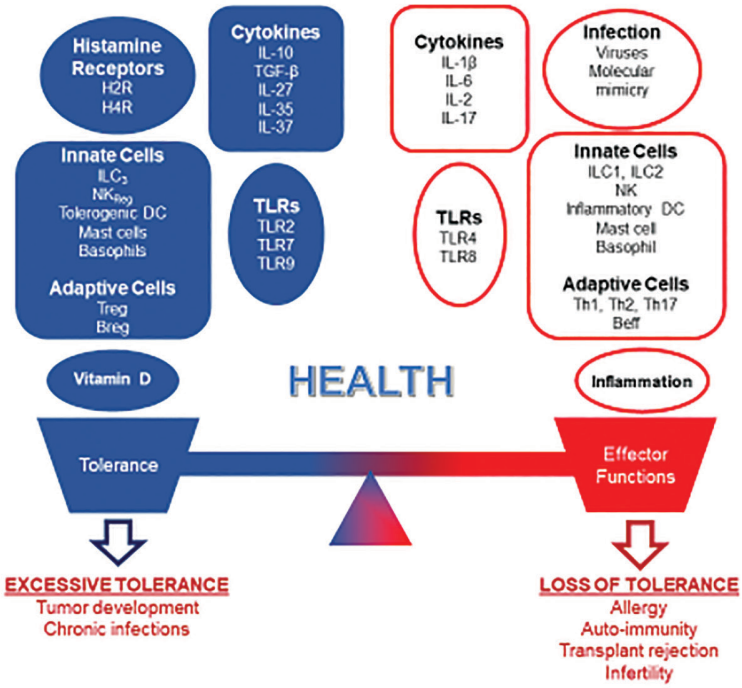

Figure 2. Contribution of Immunity to the Tolerant State.

cytokines as IL-1β, IL-6 and IL-17, and initiates inflammation. TLR2 triggering supports a tolerant state, binding of histamine to H2R and H4R also have tolerant properties. Viruses and molecular mimicry of bacteria could trigger inflammation and autoimmune diseases, respectively. Vitamin D has anti-inflammatory and tolerance promoting effects. (Blue groups indicate conditions acting to form a tolerant state while red groups indicate immune-response stimulating conditions. Beff: B effector cells.)

In allergic disorders, such as AR, asthma, atopic dermatitis, food allergy and more, both IgE mediated immediate hypersensitivity and/or delayed type T-cell mediated hypersensitivity reactions to ubiquitous antigens are observed (3, 24, 129). This may be accepted as a state of inability to tolerate these antigens, to which healthy individuals are normally non-responsive. As central tolerance mechanisms elicit discrimination of self and non-self, peripheral tolerance mechanisms generally shape the immune response with specificity to antigens and allergens. This is especially marked in allergic disorders, in which tolerance to allergens is not sufficient. In healthy individuals, regulatory T cells control and regulate both self-reactive and non-self-reactive T cell populations and prevent development of potential allergic disorders. Although allergen specific tolerance is a long-lasting state in healthy non-allergic individuals, sometimes, this stability may be disrupted by the activation of innate immune system such as by viral infections, which may lead to loss of peripheral tolerance (130). In the case of recognition of microbial particulates by TLR4 and TLR8, once triggered, these receptors induce inflammatory responses. In addition to this stimulation, in the presence of pro-inflammatory cytokines, IL-1β and IL-6 in the micro-milieu, proliferation of allergen specific $CD4^+$ T cells is triggered, which could lead to the loss of peripheral allergen specific tolerance (72).

Besides, loss of immunological tolerance to self-antigens is known as autoimmunity. Systemic lupus erythematosus, celiac disease, diabetes mellitus type 1 and multiple sclerosis are the leading examples of autoimmune disorders. The major pathologic mechanisms observed in these diseases

can be explained by T cell bypass due to superantigen production of polyclonal activation of B cells, especially by infections. Furthermore, in T cell-B cell discordance, aberrant B cell receptor mediated feedback results in perpetuating autoreactive B cells, which can also cause autoimmunity (131). Molecular mimicry is another presumed mechanism observed in autoimmunity, in which an exogenous antigen, which shares structural similarities with host antigens that may direct the immune response to self (132).

Immune tolerance also contributes to the progression of tumors. Briefly, altered antigen expressions and mutated protein structures, additional to elimination failure of the immune system, as seen in excessive tolerant states, may lead to tumor development. Several metabolic enzymes and various ligands may suppress T cell activation and proliferation. Myeloid-derived suppressor cells and Treg cells have been reported to be the major components of the immune suppressive tumor micro-environment (133). Thus, in cancer research controlling tolerance and enhancing immune responses to tumors by means of immune modulation are aimed (134). The recent studies include utilization of IgE-related anti-parasitic pathways for tumor clearance and more studies are required to reveal the success of this pathway (135).

Commentary

The strength and sustainability of immune tolerance is a defining factor in a number of diseases. Excessive tolerance may lead to insufficient defense against pathogens and may promote chronic infections as well as cancer. In contrast, from the clinical point of view, attenuated immune tolerance may manifest as allergic disorders, autoimmunity, organ transplant rejection and repeated miscarriages causing infertility.

The integrative mechanisms of central and peripheral tolerances work for achievement of a state of immune homeostasis for both the survival and the health of the organism. In the above-mentioned clinical conditions, which are presumed to be due to imbalances in immune tolerance mechanisms, novel treatment approaches that aim to warrant restoring this imbalance may provide a curative treatment option. Customization of treatment by means of precision medicine methods will enable better patient selection who will potentially benefit from personalized treatments (24, 136, 137). AIT, as an example of precision medicine approach in allergy, is the most acceptable way of induction of peripheral tolerance against the sensitizing allergens responsible for the signs and symptoms seen in allergic disorders (2, 138). In autoimmunity, although most of the patients are treated with immunosuppressive therapeutics in a non-specific manner, it is essential to induce the antigen-specific immunological tolerance to self or allogeneic antigens, while sustaining the entire immunity (139). Personalized strategies that target antigen presentation to T cells and implementing antigen-specific changes at the epitope and T cell receptor levels may provide novel insights into the management of autoimmune disorders and transplant rejections. Better understanding of the relationship between innate immunity and immune tolerance is a prerequisite for development of novel therapeutic options. The final response to a particular antigen/allergen can be defined by interactions between the environment and the immune system. As initial response to an environmental stimulus is driven by innate immunity, better understanding of immune tolerance mechanisms deeply relies on deeper knowledge on environmental stimuli and their innate recognition. Therefore, studies focused on exposome as well as microbiome that are gaining attendance nowadays will potentially contribute to this area. One should keep in mind that adaptive immunity that is responsible for both establishment and maintenance of a long-lasting immune responsiveness is mainly fine-tuned by actions of innate immunity. DCs form links between innate and adaptive arms and orchestrate immune responses differentially for each particular antigen. Understanding the DC biology is of great importance both for better illumination of their roles in precise-trimming of immune responses and for possible utilization of DCs in future therapeutic interventions.

Conclusion

Immune tolerance conveys unresponsiveness of immune system to relevant and substantial immune stimuli. A healthy immune status means generating sufficient responses against both pathogens and cancer cells, while tolerating self-tissues, commensal microorganisms as well as harmless environmental antigens. As both excessive and deficient immune tolerance lead to a number of disorders, evaluation of the tolerance intensity is important. Development of biomarkers that will reveal tolerance status is of great importance. In a peremptory way, a '*balanced tolerance*' is essential for the survival.

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Childhood Asthma: Low and Middle-Income Countries Perspective

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Abstract
Our aim is to review current asthma epidemiology, achievements from the last 10 years, and persistent challenges of asthma management and control in low-middle income countries (LMICs). Despite global efforts, asthma continues to be an important public health problem worldwide, particularly in poorly resourced settings. Several epidemiological studies in the last decades have shown significant variability in the prevalence of asthma globally, but generally a marked increase in LMICs resulting in significant morbidity and mortality. Poverty, air pollution, climate change, exposure to indoor allergens, urbanization and diet are some of the factors that contribute to inadequate control and poor outcomes in developing countries. Although asthma guidelines have been developed to raise awareness and improve asthma diagnosis and treatment, problems with underdiagnosis and undertreatment are still common. In addition, important social, financial, cultural and healthcare barriers are common obstacles in LMICs in achieving control. Given the high burden of asthma in these countries, adaptation and implementation of national asthma guidelines tailored to local needs should be a public health priority. Governmental commitment, education, better health system infrastructure, access to care and effective asthma medications are the cornerstone of achieving success. **Conclusion.** Asthma poses significant challenges to LMICs. Whilst there are ongoing efforts in improving asthma diagnosis and decreasing asthma burden in LMICs; reasons for inadequate asthma control are also common and difficult to tackle. Improving asthma diagnosis, access to appropriate treatment and decreasing risk factors should be key goals to reduce asthma morbidity and mortality worldwide.

Key Words: Low-Middle Income Countries ▪ Asthma ▪ Asthma Guidelines ▪ Children.

Introduction

Asthma is one of the most common chronic diseases in children and adults, leading to significant morbidity and mortality worldwide (1, 2). The World Health Organization (WHO) estimates that more than 300 million people currently suffer from asthma. The Global Burden of Disease estimated that asthma is the 15th highest ranked cause of Years Lived with Disability (3). Epidemiological studies in the last 30 years such as the International Study of Asthma and Allergies in Childhood (ISAAC), showed a marked increase in the prevalence of childhood asthma worldwide over the last few decades, particularly in low- and middle-income countries (LMICs) (4). Striking observation from these studies include large geographical variations, and the high prevalence reported from some centres in Africa, Latin America and Asia, which equalled that in high-income countries (HICs) such as UK or Australia. Like many other chronic diseases, asthma results from complex gene-environment interactions (5), and the diversity of genetic and environmental exposures between populations may explain the heterogeneity in the prevalence, phenotypes, and severity of asthma around the world.

It is increasingly recognized that asthma is not a single disease, but a complex heterogeneous condition in which clinical presentation may vary depending on different pathophysiological mechanisms that are associated with diverse ge-

netic backgrounds (6-8). It adversely affects quality of life of patients, may limit daytime activity, sleep, school and work attendance, and impacts upon children, their families, healthcare systems and the society. Preventable asthma deaths are still occurring due to a lack of (or inappropriate) asthma management, and in many cases the fatal outcome is associated with avoidable factors (9). Unfortunately, the burden of the disease is higher in low-middle income countries (LMICs) and in underserved populations in HICs (10). For example, a recent large study in the US has highlighted that mortality continues to be higher in African American women (11), and we need to better understand these disparities to develop accurate risk-prediction tools (12).

Asthma in LMICs is not only rising, but is becoming a major public health problem in countries where infectious disease and other health priorities often predominate. Extreme social inequalities, poor access to medical services, poor health education, lack of access to basic infrastructure, fast modernization and ´urbanization´ of rural environment, increasing air pollution, smoking habits and change in the diet are some of the factors associated with the rise of asthma. Low accessibility to basic medications, weak healthcare services, poor adherence with prescribed therapy, lack of asthma education, and social and cultural factors have been proposed causes for the lack of control of the disease (13).

In this article which focusses on LMICs, we review asthma epidemiology, achievements from the last 10 years, and persistent challenges of asthma management, and discuss what is needed to improve asthma control, prevent avoidable deaths and decrease the burden of disease.

Epidemiology of Asthma in LMICs

Asthma Prevalence

Most of the information about the prevalence of asthma worldwide originates from a series of repeated cross-sectional studies of the prevalence of asthma symptoms and other allergic diseases (e.g. rhinitis and eczema) (1). One of the largest and most comprehensive studies in children was the ISAAC study, which demonstrated a striking variation in the prevalence of asthma symptoms between different countries, and between different centres within the same country (14, 15). The ISAAC Phase Three included more than 1.2 million children from 98 countries in all WHO regions, with reported prevalence of asthma in 6-7-year-old children ranging from 2.4% in Jodhpur, India to a staggering 37.6% in Costa Rica (14, 15). In some countries the prevalence increased, in others it plateaued, whilst in some the numbers of affected children decreased. For example, the prevalence had increased by 0.16% per year in Africa, 0.32% per year in Latin America, and 0.07% per year in the Asia/Pacific region, and had decreased by 0.07% per year in Western Europe (14, 15).

Another important finding from ISAAC surveys was the increased prevalence of severe asthma in LMICs, with substantial morbidity and economic costs (15). These findings were confirmed in other studies. For example, a recent study from Uganda, found that in a cohort of 449 children and adults with asthma who were followed for two years, 59.6% of patients with asthma experienced at least one exacerbation in a year, while almost a third experienced three or more exacerbations in a year (16). In Senegal, Hooper and colleagues (17) reported that 62% of children with asthma had severe symptoms. These studies demonstrated an unacceptable level of poor outcomes among patients with asthma in some LMICs and urged for a change.

One issue to take into account is that many factors influence the clinical diagnosis of asthma in different populations. For example, definition of asthma might not be the same everywhere, and the awareness of asthma symptoms, medical training, experience, cultural and social factors may differ as well. Further efforts are currently being made to continue monitoring asthma prevalence and severity globally for both children and adults. For example, the Global Asthma Network (GAN) (http://www.globalasthmanetwork.org/) was established in 2012 to regularly collect data on

asthma prevalence, severity and risk factors. GAN phase I is expected to report the current prevalence of asthma symptoms worldwide by 2020.

Asthma Mortality

The Global Burden of Disease estimated that 420,000 deaths occurred globally from asthma in 2016 (2). Although asthma prevalence is higher in HICs, most asthma-related mortality occurs in LMICs. Also, it should be noted that there are differences in mortality time trends between adults and children. For example, in the United States, the overall asthma-related mortality has declined from 1999 to 2015, but the mortality rate among children aged 1-14 years has not changed (11). Unfortunately, information is scarce on paediatric asthma mortality in LMICs.

In a recent analysis which included children and adults from 46 countries (mainly high- and middle-income), the estimated global asthma mortality rates did not change over the past decade (10). The important finding of this study was that asthma mortality significantly reduced from 1993 to 2006 (0.44 to 0.19 deaths per 100000 people), but there were no further improvements since 2006, with the global asthma mortality remaining unchanged. As reported by others, it is likely that the reduction in asthma mortality in the 1990s through the 2000s was primarily due to the increase in use of the anti-inflammatory asthma treatments (particularly inhaled corticosteroids) (18). However, it is of concern that in the last 15 years, no further gains have been made.

Environmental Exposures and Other Risk Factors Associated with Poor Asthma Outcomes in Children in LMICs

Variability in the prevalence of asthma between countries and the increasing prevalence in the last few decades suggest that environmental exposures have an important role on asthma occurrence (19). Many risk factors which are amenable to intervention have been identified within LMICs, including active smoking and environmental tobacco smoke (ETS) exposure, indoor and outdoor air pollution (such as biomass fuel), allergen exposure and overuse of antibiotics (20-23). Moreover, other factors related to poverty, including high-risk conduct exposures, early respiratory viral infections, dietary factors and urbanization may be determinants of the trends of increased asthma prevalence. For example, in an ecological study in Ecuador, Rodriguez et al. (23) showed that prevalence of asthma increased with increasing levels of urbanization. However, data from Ghana suggest that rather than urban living *per se*, it is affluence and westernized lifestyle that are associated with higher risk (24-26). In Brazil, better asthma outcomes (reduced hospital admission and mortality) were associated with living in an urban environment, possibly due to the better access to health care and free ICS supply (27). Although this rural-urban gradient in asthma remains poorly understood, it could be due to levels of outdoor and indoor pollution, microbial or parasitic infections, or changes in lifestyle (e.g. diet).

Indoor Air Pollution

WHO estimates that more than 3 billion people rely on solid fuels as a source of energy, contributing to indoor air pollution. Passive exposure to tobacco smoke and indoor biomass combustion are considered the most important sources of indoor pollution which may be relevant to the development of childhood asthma. Prevalence of ETS exposure in developing countries varies significantly from 10 to 60%. In most countries, active smoking is common and may be due to cultural factors, lack of regulation and poor law enforcement. In utero maternal smoking and postnatal exposures to cigarette smoking are associated with asthma development and asthma morbidity in childhood (28). Coal and wood combustion indoors have been reported to increase the risk of upper and lower respiratory infections in infants and preschool children, and may also be associated with increased asthma prevalence (2). Analysis of global ISAAC data reported an association between open-fire

cooking and asthma symptoms in both children aged 6-7 years and those aged 13-14 years (29). In Guatemala, indigenous children exposed to open fire for cooking had a higher prevalence and severity of asthma symptoms (30).

Outdoor Air Pollution

Outdoor pollution from motor vehicles, very common in urban areas, is associated with persistent respiratory symptoms and higher prevalence of asthma (31-34). A study in Puerto Rico showed joint detrimental effects of vitamin D deficiency and traffic-related air pollution on severe asthma exacerbations (35). In a recent meta-analysis, Orellano *et al* found that living in a polluted environment in Latin America and the Caribbean was associated with higher rates of asthma in children (31). In 2019, several large studies linked air pollution to poor lung function (36), increased risk of asthma deaths (37) and high risk of asthma development (38). A study in China on >7,000 asthma deaths between 2013 and 2018 found that short-term exposures to fine particulate matter <2.5 mm in diameter ($PM_{2.5}$), NO_2, and O_3 increased mortality (37). Among asthmatic children in Peru, air pollution adversely affected asthma control and quality of life (39, 40). Finally, perinatal exposure to ambient ultrafine particles (<0.1 mm diameter) has been linked to the onset of asthma in children (independent of $PM_{2.5}$ and NO_2) (38). Air pollution may interact with other indoor environmental exposures. For example, ambient air pollutants and indoor endotoxin exposure act synergistically to increase increased emergency room visits for asthma in both children and adults (41). Importantly, the adverse health effects were apparent at concentrations below current standards in HICs, suggesting that air quality standards will need to be strengthened if we are to protect patients with chronic lung diseases (42), and emphasise the need for developing and implementing effective policy measures in LMICS, which are most affected by rising pollution (12, 43).

Allergen Exposure

Although sometimes considered a less important contributing factor for asthma prevalence in some regions of LMICs (44), atopy is high in most Latin American countries. Using data from phase two of the ISAAC study, Weinmayr et al. (44) found that the association between current wheeze and skin prick test positivity was statistically significant in both affluent (OR 4.0, 95%CI 3.5-4.6) and non-affluent (OR 2.2, 95%CI 1.5-3.3) countries. Exposure to common allergens is one of the triggers for developing asthma symptoms, but its relationship to asthma development is unclear (45). Allergens originate from a wide range of sources, including house dust and storage mites, animals, indoor moulds and cockroaches. Exposure to indoor allergens has been associated with asthma symptoms, airway hyperresponsiveness and severe asthma exacerbations in several cross-sectional studies in LMICs (46, 47). In a study performed in Costa Rica, we studied 403 asthmatics (ages between 6 and 14 years) who were carefully assessed using symptom questionnaires, spirometry, measurements of serum total and allergen-specific IgE, peripheral blood eosinophil count, and body mass index, and the assessment of airway responsiveness to methacholine. In a multivariate analysis, parental report of mould exposure in the house, low FEV1/FVC ratio, and a positive IgE response to house dust mite were strongly associated with airway hyperresponsiveness to methacholine (46). In a cross-sectional study in Puerto Rico, Blatter et al. (48) found that among children with asthma, those with the highest exposure to glucan (a component of fungal cell wall) had much greater odds of one or more visits to emergency room for acute asthma symptoms (OR 8.7, 95%CI 2.7-28.4).

Diet and Obesity

Changes in diet and increasing obesity have been related with the increase of asthma prevalence worldwide. Vitamin D deficiency has been associated with asthma in several LMICs (49, 50). Among 616 school-aged Costa Ricans with asth-

ma, low vitamin D level (28% of children with levels of vitamin D <30 ng/ml), was associated with increased odds of asthma-related emergency department visits or hospitalizations in the previous year (50). This was also the first epidemiological study to demonstrate an association between low vitamin D levels and increased serum IgE and eosinophil counts (50). Other studies which focused on dietary patterns have shown that fast food or a "Western" dietary pattern is associated with higher risk of asthma presence, and also asthma severity. Overweight and obesity are common in LMICs, particularly in Latin America, where recent studies suggested a prevalence between 16-36% among children (51). Similar to HICs, overweight and obesity in LMICs have been associated with asthma presence and severity (52, 53).

Socioeconomic Factors

Socioeconomic factors and poverty play important role in asthma control. Among children of different Latino ethnicities, girls from low-income families had lower adherence to inhaled corticosteroids (ICS) over a 12-month period, and Puerto Rican children had poorer outcomes than Mexican (54, 55). Worryingly, less than a quarter of children were adherent with treatment. Low income and other social determinants result in poor access to care, low accessibility to essential basic therapy, and wide disparities in health care (56). However, additional issues besides access to healthcare and availability of asthma medications may contribute to high asthma burden in underserved populations, including psychosocial stressors, behavioural risk factors, poor medication adherence, underuse of primary care providers, but overuse of emergency health services, increased school and work absenteeism and increase in general morbidity and mortality (57, 58). For example in a cohort of 98 children from low income families in Brazilian rural area, Jentzsch et al. (59) showed that compliance to recommendations to reduce or minimize allergen and/or trigger factor exposure was achieved in less than 9.2% of patients. Moreover, in HIC, socially disadvantage paediatric patients have elevated asthma morbidity as a result of socio-economic status, minority affiliation, psychosocial stress and high adverse environmental exposures (60).

Managing asthma in poor or disadvantaged population can be challenging and is often associated with worse outcomes. However, interventions to address some modifiable factors in these population are possible and have had good results. Examples of these interventions include asthma education and self-management, environmental control in the home, culture-specific asthma programs, school-based programs, home visits by health workers (e.g. nurses, doctors, social workers), tobacco smoke cessation or a combination of the above (18, 61).

Difficulties in Asthma Care in LMICs

It has been recognized that management of asthma is a difficult task in HICs, and even more so in LMICs. As most other common non-communicable diseases, asthma represents a challenge to public health because of its high prevalence, increasing severity, projected trends, and economic burden. The financial impact for persons with asthma and their families, as well as for healthcare systems and governments, is very high. At the same time, most LMICs face communicable diseases such as pneumonia, tuberculosis, dengue, HIV and other infectious diseases.

Amongst many obstacles to good disease control, we wish to highlight the lack of education about asthma, issues related to medication availability and delivery, cultural barriers, and low priority by health authorities. Global asthma guidelines have played an important role in raising the awareness of childhood asthma and improving diagnosis and management in LMICs. However, most asthma guidelines are long and complex, making them difficult to implement in the healthcare systems already overstretched by the pressure of communicable diseases. To be effective in reducing the burden of asthma, guidelines must be relevant to the specific population, must be culturally acceptable, and must be adapted to each health

system formalized with political engagement and commitment. Various national and/or regional asthma strategies in LMICs have emphasised aspects which are of relevance to specific areas or countries, including the medication access and affordability, specific environmental exposures, lack of asthma education, co-morbidities, and psychological problems (62).

Similar to other chronic diseases such hypertension and diabetes; asthma control is not achieved in most patients, despite available management guidelines. A survey performed in 11 countries in Latin America showed that only 2.4% of patients met all of the GINA criteria for total asthma control, suggesting under-recognition of uncontrolled asthma, underuse of appropriate controller treatment, inadequate patient education, and patient denial as possible explanations (63). Since the development of worldwide guidelines on the diagnosis and management of asthma, special attention on achieving and maintaining asthma control as the key goal in asthma treatment has been a priority. In clinical studies of children with asthma in LMICs, the most cost-effective intervention for improving asthma control and preventing acute exacerbations is emphasis on the use and availability of controller medications, such as ICS for mild asthma, with the addition of long-acting β-2 agonist for moderate asthma (18).

Comprehensive approaches such as developing models for highly cost-effective, affordable and feasible interventions are critical for asthma control. Unfortunately, there is limited information on the impact of population-based interventions for asthma control, but some studies from LMICs indicate it may be cost-effective. Public health efforts have improved management in some countries. In Brazil, an ecologic study showed that municipalities which were offering free medication for asthma had lower hospital admission rates and lower mortality from asthma (64). In a study in Costa Rica, a marked decrement in hospitalizations (53%) and mortality (80%) was seen following implementation of the National Asthma Plan (NAP) and the use of beclomethasone as an affordable preventive medication (18). This NAP consisted of education meetings at all major public (both urban and rural) healthcare centres, which facilitated early diagnosis, implementation of non-pharmacological measures (smoking ban, adherence, reduction of indoor allergens), early treatment using ICS as first-line therapy for asthma control, the use of spacers, early use of reliever medication to treat exacerbations, appropriate referral to specialists for asthma care, and avoidance of common allergen sources (e.g. dust-mite and cockroaches) or tobacco smoke.

Despite undeniable fact that effective national asthma strategy results in a reduction of the burden of asthma on society as a whole, many countries lack such program. This problem has been highlighted by the recent email survey of investigators from 112 countries which participate in the Global Asthma Network. Of the 112 countries, only 26 (23.3%) had a national plan for children, 24 (21.4%) had a national asthma plan for adults, and 22 (19.6%) countries had a strategy for both children and adults. Unfortunately, the proportion of LMICs with a strategy was lower than that in high-income countries (62).

Development and Implementation of Asthma Guidelines in LMICs

The development and implementation of asthma treatment guidelines can be expensive and challenging. Figure 1 illustrate most components that should be included in an asthma strategy to achieve better outcomes. Guidelines should include realistic recommendations on how to improve early detection of asthma symptoms, control of environmental risk factors, and provide mechanisms of access to effective anti-inflammatory treatment.

Many obstacles are faced by LMICs in implementing asthma guidelines. Table 1 lists most barriers that have to be overcome by these countries in order to achieve better outcomes and decrease the economic and social burden of asthma. Although some problems may be unique to particular countries, most LMICs share similar barriers to asthma management. Thus, collaborative public health and research efforts could have a major impact at

a regional level. Such efforts should include vigorous campaigns to control and eliminate tobacco use, asthma education and non-pharmacological interventions (e.g. replacing biomass fuels, reducing allergen exposure, psychological support and weight loss).

One of the most important issues is the availability and affordability of essential asthma medications. Optimal asthma care requires universal access to medications, and yet many asthmatics in LMICs or in the underserved population in HICs have difficulty accessing necessary medications. In a cross-sectional study conducted in 52 countries, huge variability was found in affordability across countries. In this study, the availability of ICS was particularly low, with some countries subsidizing the asthma medicines thereby making them free or less expensive to patients, but other increasing their price. Many patients have only access to a bronchodilators, and those who may benefit from affordable ICS such as beclomethasone, can spend between half a day´s wages to even 14-days wage for one inhaler device (65). We would argue that cheap inhaled anti-inflammatory agents (e.g. beclomethasone) should be available free of charge for children with asthma in LMICs.

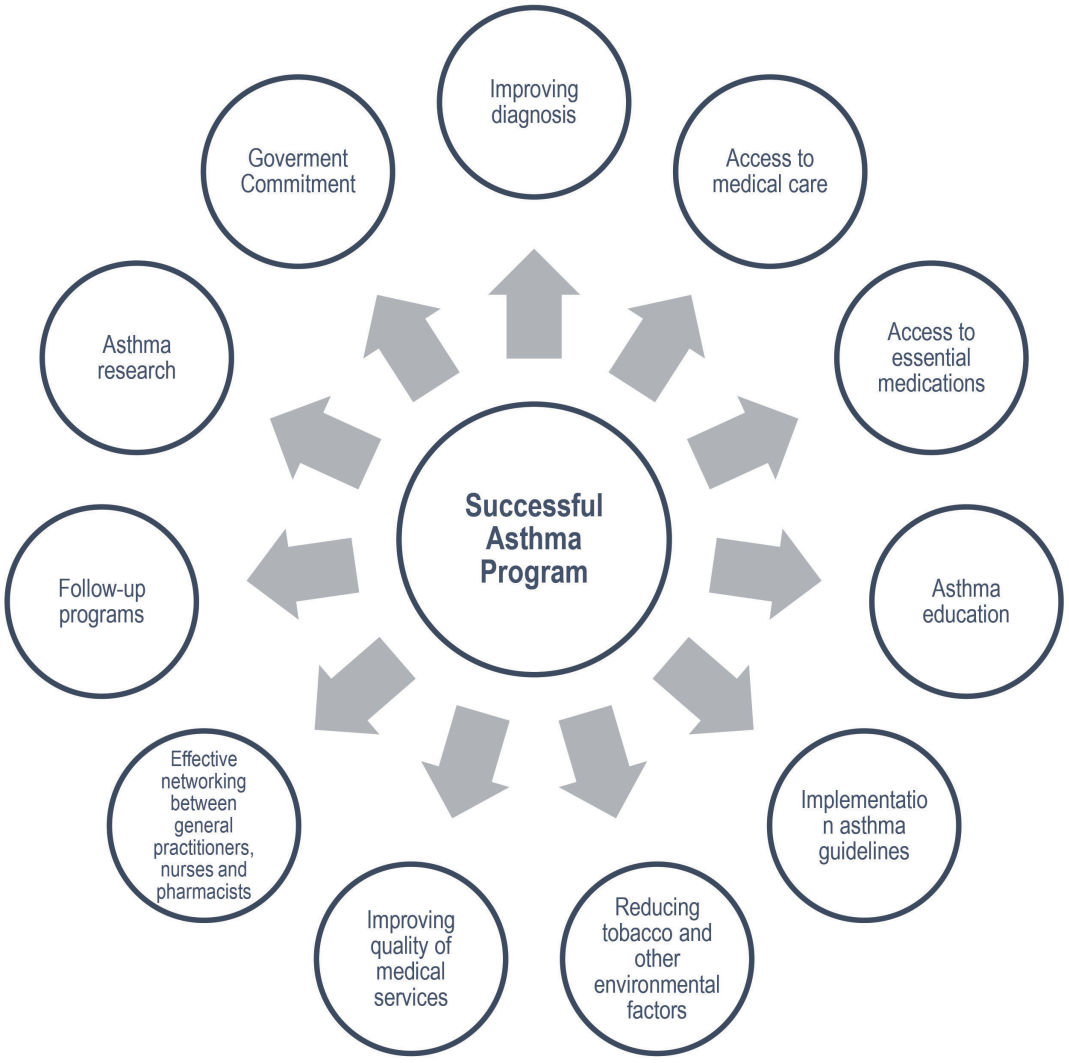

Figure 1. Components of a Successful Asthma Program; Adapted from (66, 67).

Table 1. Barriers for Asthma Control and National Asthma Strategies Implementation Adapted from The Global Asthma Network Report 2018 (2)

Disease inherent barriers
Underdiagnosis
Undertreatment or inadequate treatment
Lack of adherence
Environmental
Air pollution (both indoor and outdoor pollution)
Tobacco smoke
Occupational triggers
Healthcare system
Low public health priority
Low rates of strategies dissemination
Lack or weak health system infrastructure
Poor access and distribution of asthma medications
Inadequate governmental resources for asthma care
Lack of continuing medical education and training systems
Patient barriers
Lack of information
Over-reliance on acute medication (e.g. SABA)
Use of unproven or alternative therapies
Cultural and social problems and beliefs
Medication beliefs

Conclusions

Asthma burden in LMICs is high and continues to increase. Whilst there are ongoing efforts to developing novel (often expensive) therapies targeting patients in high-income countries, a great impact can be achieved by improving asthma control, preventing asthma attacks, and decreasing asthma burden in LMICs. Reasons for inadequate asthma control in underserved populations include low accessibility of effective controller medications, weak infrastructure of health services for the management of chronic diseases, poor adherence to therapy, lack of educational approaches, persistent exposure to risk factors and social, cultural and language barriers. Improving access to appropriate treatment including ICS, salbutamol and spacers should be a key goal to reduce asthma morbidity and mortality worldwide.

Conflict of Interest: The authors declare that they have no conflict of interest.

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RSV Lower Respiratory Tract Illness in Infants of Low- and Middle-income Countries

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Abstract
This review addresses differences in respiratory syncytial virus (RSV) lower respiratory tract illness (LRTI) between industrialized and developing countries and provides observations associated with the dissimilar consequences of viral infection in both environments. RSV LRTI is an important cause of morbidity and mortality in infants worldwide. Its burden is highest in developing countries, where most hospitalizations and mortality occur. Palivizumab has been approved for disease prevention in premature infants in numerous countries but its cost and requirement for several doses hampers its routine use. The significant gap between low- and high-income countries in mortality rates stresses the need to identify specific risk factors for RSV LRTI prevention in different populations. **Conclusion.** RSV LTRI continues to be a serious problem for industrialised and developing countries, although mortality occurs preferentially in the latter. Several vaccines and monoclonal antibodies to prevent severe disease are advancing steadily in late phase trials. The next decade may witness a change in the landscape of RSV infections in young infants.

Key Words: RSV ▪ Global Burden ▪ Developed Countries ▪ Risk Factors.

Introduction

Lower respiratory tract infections (LRTI) are the main cause of morbidity and mortality in infants and young children between 1 month and 5 years of age (1). Respiratory syncytial virus (RSV) is the most frequent cause of LRTI in infants, associated with 33.8 million new episodes of RSV LRTI every year worldwide, and an incidence in developing countries doubling that of industrialised nations. Worldwide, RSV is associated with about 28% of all LRTI episodes and 13-22% of al LRTI mortality in young children (2).

In 2005, 3.4 million children developed severe episodes of RSV LRTI requiring hospital admission, particularly in infants younger than 6 months. Global mortality is estimated to range between 66,000 and 199,000 deaths per year; 99% of these deaths occur in developing countries (3). Conversely, RSV LRTI deaths in industrialized countries are infrequent and affect high-risk infants with chronic lung, heart, or neuromuscular disease, children with Down's syndrome and those born prematurely (4) (200 deaths per year in children between 0–59 months vs 47,800–74,300 deaths in developing countries) (2).

Acute RSV bronchiolitis is a seasonal disease, typically starting in late fall until early spring, with a winter peak. Infection is often mild and presents with symptoms that mimic a common cold, but can progress to display tachypnea, wheezing, and crackles, in the most severe cases leading to respiratory failure and/or death. In addition to deaths at medical institutions, in developing countries approximately half of all RSV deaths in infants and young children occur at home (5). Risk factors for home death in this population associate with environmental and social variables including precarious household conditions, crowding, incom-

plete vaccination for age, no breastfeeding, and prematurity (6, 7). In addition to acute disease, infants who experience severe RSV LRTI early in life are more prone to develop wheezing during early childhood, and may have increased rates of airways hyperreactivity and asthma later on (8-11). Therefore, preventing severe RSV LRTI may impact the global burden of paediatric wheezing. New vaccines and monoclonal antibodies (mAb) against RSV are promising strategies to reduce the burden of acute and long-term illness associated with the virus.

The aim of this review is to address disparities in RSV LTRI burden and disease outcome between industrialised and developing countries.

Severe Disease Worldwide

The incidence of severe RSV LTRI differs between developing and industrialised countries. In 2015, the number of episodes of severe RSV LTRI in developing countries was 6.1 million vs 212,000 in industrialized countries. Hospital admissions showed a similar difference, 2.6 million vs 344,000 hospitalizations, respectively (2). In children <6 months of age, rates exhibit logarithmic differences between developed and developing nations: 36.1 vs 3.2 per 1,000 children per year. Rates in 0 to 59 months old also differ from 10.2 to 3 per 1,000 children per year (2). Most episodes of severe RSV LTRI occur during the first 6 months of life, when infants experience the highest hospitalization rates (20.1 per 1,000 infants per year) (12). Rates are increased among preterm infants to 63.8 per 1,000 per year (12). In developing countries, the hospital case fatality rate (hCFR) is 2.2 for infants <6 months of age, and 2.4 in those between 6 and 11 months.

Socioeconomic factors affect the risk of experiencing respiratory failure due to RSV LTRI. Increased risk is observed in infants living in homes with no sewage or exposed to indoor smoke (13). In addition, medical practice in impoverished areas of the world also affects the odds for survival. RSV mortality in a low-income region of Argentina strongly associated with the development of clinically-significant pneumothorax during hospitalization (13). Rate of pneumothorax in these intensive care units was several-fold higher than those reported in association with RSV LRTI in industrialized countries (14). In this same low-income region, precarious household conditions including homes made of tin or wood with a dirt floor (OR 2.30), no running water (OR 6.14) and crowding (OR 3.54) increased the risk for at-home death. Additional socioeconomic risk factors for mortality included being born to a young mother (<19 years old) and incomplete vaccination for age (6). In these situations, parents often experience deficient interactions with the healthcare system before their children die. It is not infrequent to encounter fatal cases with a history of incomplete prenatal healthcare. Evidently, beyond the offending agent itself, the home environment of babies and parental communication with the health care system are important determinants of the children's chances of survival (6).

Recurrent Wheeze and Asthma

Severe RSV LRTI in early life is associated with an increased risk of recurrent wheeze and asthma (8-11). A longitudinal study that followed children <1 year hospitalized with RSV-LTRI up to 18 years of age showed an increased prevalence of asthma/recurrent wheeze (39% vs 9%), clinical allergy (43% vs 17%) and sensitization to perennial allergens (41% vs 14%) in the RSV cohort compared with controls (9). Furthermore, a second cohort study reported that 31% of children with early childhood asthma had clinically significant bronchiolitis during infancy, with a prevalence of asthma among hospitalized children due to bronchiolitis of 22% (10). In fact, birth before the winter virus peak has been shown to associate with a greater risk of developing asthma in later life (15). Infants born 4 months before the virus peak had 29% more risk of developing asthma compared to infant birth 12 months before the peak (16).

Interventional studies are most informative in defining the role of RSV in long-term wheezing. A double-blind, placebo-controlled trial with palivi-

zumab (a monoclonal antibody targeted against the RSV neutralizing fusion protein) in healthy preterm infants in The Netherlands, resulted in a 61% reduction of wheezing days and 10% reduction in recurrent wheeze, 11% in infants that received the mAb vs 21% in those that received placebo (8). Interestingly, a similar study with a high-affinity mAb against RSV (motavizumab) in healthy Native Americans born at term in Arizona had no effect on rates of medically attended wheezing in 1- to 3-year-old children despite preventing severe acute RSV LRTI.

Many of these studies reported follow-ups at the age of 6 years. In the Dutch study, palivizumab significantly reduced parent-reported asthma on the basis of different rates in infrequent wheezing (one to three episodes per year) when compared with placebo recipients, but physician-diagnosed asthma and lung function did not differ between groups (16, 17). In a second study in Japan, palivizumab prophylaxis failed to suppress atopic asthma but reduced recurrent wheezing. Evidently, ongoing vaccine and monoclonal antibodies phase 3 studies are a superb opportunity to address these questions and define the precise role of RSV in asthma inception. Yet asthma is a set of heterogeneous conditions with different molecular mechanisms of illness but common symptoms. Therefore, it is likely that preventing severe RSV LRTI will modulate one or a few of these "asthmas" but fail to alter others.

RSV and All-Cause Pneumonia

Today, there is enough evidence to support conducting a detailed evaluation of the role of RSV vaccines and monoclonal antibodies in modulating the burden of all-cause severe LRTI and pneumonia months after immunization and after the RSV season. Recent observations suggest that interventions may have a greater-than-expected impact on long term mortality given the aforementioned effects, and -through these unclear mechanisms- contribute to modulate the burden of lung disease overall, with potential long-term consequences in lung function.

Coinfections

Multiplex polymerase chain reactions (PCR) often detect other viruses combined with RSV showing that coinfection with more than one respiratory virus is frequent in children (18). Yet, the clinical implications of more than one infectious virus in infants with LRTI remain unclear. Several studies suggest that respiratory virus co-infections may increase severity of RSV disease (19-21), reporting infants with longer hospital stays and a greater need for supplemental oxygen; however, other studies do not confirm these results (22, 23). The frequency of co-infection with other viruses in RSV LRTI varies considerably in different studies, ranging from 11% to 56% (19, 22). This may be affected by the population composition in different studies, seasonal variation of respiratory viruses, and the breadth of pathogens explored in each report.

Disease Prevention

Palivizumab, a humanized monoclonal antibody targeting the fusion protein, the main neutralizing antigen in RSV, is the only licensed intervention to prevent severe RSV LRTI. Palivizumab prevents 55% hospitalizations in high risk preterm infants, and decreases the total days of hospitalization, oxygen supplementation, and intensive care (24). The antibody is used in high-risk preterm populations in high and middle-income countries (25, 26). Unfortunately, due to its high cost, its requirement for four to five injections per child per season, and the challenge of defining the exact timing and duration of the RSV season in subtropical and tropical regions, its adequate use in the developing world is infrequent. Another important strategy for RSV disease prevention is breastfeeding, and its effectiveness has been extensively documented.

Motavizumab, a second generation humanized monoclonal antibody with higher affinity for RSV than palivizumab, was studied in children in Navajo and Apache reservations (27). This population routinely experiences high rates of severe RSV disease. Motavizumab showed an 87% relative reduction in the proportion of hospitalizations but

elicited skin reactions that precluded its use (28). Newer mAbs have been undergoing evaluation in recent years. Suptavumab (REGN2222), a mAb of extended half-life against site V in RSV F, did not meet its primary efficacy endpoint in preterm infants (NCT02325791). Despite protecting against disease caused by RSV subgroup A, an unexpected mutation in site V of RSV F in circulating RSV B viruses affected its overall efficacy. Medimmune, the manufacturer of palivizumab, is now undertaking phase 3 studies with MEDI8897, which presents amino acid substitutions in the Fc region (YTE technology) that enhance binding to the MHC class I-neonatal Fc receptor and extend its half-life to 85 to 117 days. The mAb targets antigenic site Φ in the pre-fusion conformation of RSV F. Another mAb of extended half-life advancing into late phases of clinical evaluation is MK-1654 from Merck (NCT03524118) targeting RSV F site IV.

RSV vaccine development has been a sustained goal since the 1960s, when a formalin-inactivated RSV (FIRSV) vaccine was used to immunize children and elicited a non-protective and disease-enhancing response (29, 30). After RSV exposure, two immunized infants died and 80% of those infected with RSV were hospitalized. FIRSV associated with non-neutralizing, low-avidity anti-F IgG responses and a Th2 bias of the cytokine and cellular response (31, 32). Natural immunity, instead, presents innate responses with polymorphonuclear and mononuclear cells and type I and III interferon (33-35).

Antibodies, as demonstrated with palivizumab and a polyclonal immune globulin enriched for RSV antibodies (Respigam), are able to prevent severe RSV LRTI. RSV infection elicits polyclonal, high avidity, neutralizing antibody responses against RSV F (36), which is highly conserved between RSV subgroups A and B (37). The RSV attachment (G) protein also elicits polyclonal neutralizing responses but presents increased variability between subgroups. T cell responses are also important for protection against RSV, as $CD8^+$ T lymphocytes clear the virus from infected cells and $CD4^+$ T lymphocytes contribute to orchestrate T-dependent antibody responses (38).

Several studies demonstrated an association between severe illness in young infants with low concentrations of cord-blood RSV antibody (39, 42). Chu et al. showed that infants with higher cord blood antibody titres are at lower risk of infection during the first 72 weeks of life (43). And a second study showed the risk of hospitalization in the first 6 months to be inversely correlated with concentrations of cord-blood neutralizing antibody (44). Therefore, immunization during pregnancy aims to protect the infant in these first few months of life through transplacental transfer of antibodies (45). Maternal immunization strategies to protect newborns and infants proved effective against influenza, pertussis and tetanus (45, 46).

Transplacental transfer of immunoglobulin concentrates in the third trimester of pregnancy. Prematurity is, therefore, associated with reduced transfer of antibodies, particularly in infants born before 28 weeks' gestation. Hence, premature infants may not benefit from a maternal immunization strategy. In a study performed by Yildiz et al., antibody transplacental transfer rates were lower in small for gestational age and large for gestational age infants in comparison to those appropriate for gestational age, but these observations have not been replicated (47, 48).

Recently, a RSV F protein nanoparticle vaccine was tested in pregnant women in a randomised, double-blind, placebo-controlled trial and demonstrated good safety and immunogenicity. Transplacental antibody transfer ranged between 90%–120%, with higher transfer efficiency noted among women immunized ≥30 days before delivery as contrasted with those immunized for <30 days (49, 50). The vaccine failed to meet its primary endpoint to reduce the rate of medically significant RSV LTRI in infants through 90 days of life, despite an overall efficacy of 39.4%. However, it was effective in protecting against RSV hospitalizations and severe disease worldwide (40% and 44% respectively) (51). Novel pre-fusion constructs from Pfizer and GSK designed for maternal immunization entered late phase trials in 2019.

Live attenuated RSV vaccines do not prime infants for enhanced RSV disease. In addition, these

vaccines are administered intranasally, needle-free and can generate an immune response even in the presence of passively acquired maternal antibodies (52). A variety of live-attenuated RSV vaccines are under development by a joint effort of Sanofi and NIH. An important challenge will be to attain an adequate balance between attenuation and immunogenicity (53, 54). Vectored based-vaccines using adenoviruses are also in clinical trials, as well a recombinant BCG expressing RSV N protein. Adenoviruses are highly immunogenic and induce both innate and adaptive immune responses, and are being investigated as vectors targeting viral, bacterial and protozoan pathogens. The chimeric candidate rBCG-N-hRSV vaccine presents the advantage that BCG induces Th1 immunity, turning the immune response away from the undesirable Th2 priming. This vaccine is expected to mainly elicit cellular immunity against RSV, which is yet to demonstrate sufficient protection against RSV.

Conclusions

The burden of RSV LRTI in infants and young children worldwide is significant. Mortality due to the virus is a serious problem in developing countries. Disparities in disease outcome between developed and developing countries might associate with differences in access to health care, health care resources, living conditions, and the high cost of preventive measures like palivizumab for high risk populations. Yet, the situation may improve in coming years. Numerous prophylactic interventions are steadily advancing in late phase trials and may provide effective solutions against the pathogen in the next decade.

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Peanut Allergy – No Longer a Life Sentence

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Abstract
In this review we provide an overview on the latest knowledge in the prevention and active management of peanut allergy. The rise in incidence of food allergy has generated new challenges in the management of affected individuals. Strategies to counteract the increase in prevalence of peanut allergy can be considered as a pyramid, beginning with primary prevention of those at risk through earlier introduction of peanut into the infant diet, to secondary prevention of peanut-sensitised children through improvements in the correct diagnosis of peanut allergy and finally to the treatment of children with proven peanut allergy. **Conclusion.** With the paradigm shift towards an active management, peanut allergy should no longer be seen as a life sentence.

Key Words: Immunotherapy ▪ Peanut Allergy ▪ Prevention.

Introduction

The rise in incidence of food allergy worldwide has generated new challenges in the management of affected individuals. However, at the same time, we are learning more about the development of food allergies and strategies to both reduce the risk and offer active management of affected patients.

In this review we provide an overview on the latest knowledge in the prevention and management of peanut allergy.

Epidemiology

Peanut allergy was uncommon before the 1990s, where reports were limited to case series or small cohorts (1, 2). The prevalence of peanut allergy has risen significantly over the last 3 decades (3-5), although this is likely to be, at least in part, due to increased knowledge and recognition of the condition. In both the United Kingdom and Australia, there is evidence that while peanut allergy increased prior to 2000, this increase has now plateaued (4, 6). A systematic review conducted in Europe between 2000 and 2012 reported the point prevalence of challenge-confirmed peanut allergy to be 0.22% (95% confidence interval (CI), 0.16–0.28), with overall pooled estimates for self-reported lifetime prevalence of peanut allergy as 1.3% (95% CI, 1.2–1.5) (7). In Australia, the prevalence of peanut allergy at 12 months was 3.0% (95% CI, 2.4–3.8) and at 4 years 1.9% (95% CI, 1.6–2.3) (8). Even though evidence for the rise in prevalence of food allergy appears to preferentially affect industrialised nations, there is growing evidence of similar trends in rapidly developing countries such as Thailand (9) and China (10).

Peanut is not only a leading cause of food-induced anaphylaxis (11) but is also a major trigger for fatal food-related anaphylaxis. A systematic review and meta-analysis estimated the incidence of fatal peanut anaphylaxis to be between 0.73 and 4.25 per million person years (12).

Risk Factors

Eczema and egg allergy are 2 known independent risk factors for peanut sensitisation and/or subsequent allergy (13-15), although egg allergy may simply be a surrogate for food allergies, given the use of egg as a weaning food in many cultures. The dual-allergen-exposure hypothesis was first described a decade ago and is now widely accepted as explaining, as least in part, the link between infant eczema and risk of subsequent food allergy (16). According to this hypothesis, the transcutaneous exposure of peanut (present in the environment) is accentuated in eczema, while the lack of oral exposure promoting oral tolerance act together to increase the risk of food allergy. Indeed, high levels of household peanut consumption by family members of infants with eczema was found to be a risk factor for peanut allergy (17). Also supporting this hypothesis was evidence showing peanut sensitisation occurring in children through the application of peanut oil to inflamed skin (13). There is a molecular basis for the increased skin permeability in eczema: loss-of-function variants of the epidermal barrier protein, filaggrin, and missense mutations in the serine peptidase inhibitor Kazal type 5 (SPINK5) skin barrier gene are both predisposing factors for eczema (16, 18) and have been associated with an increased risk for food allergy (19, 20).

In a recent US observational study, key clinical factors associated with peanut allergy in a high risk infant cohort (defined as infants between 3 to 15 months having likely egg and/ or milk allergy, and/or moderate to severe eczema with a positive skin prick test (SPT) to egg/ milk) are younger age at initial presentation, lack of breastfeeding, and greater sensitisation to peanut-specific IgE and IgE to the peanut component Ara h 2 (21).

Natural History

Peanut allergy is persistent in around 80% of children (22-24), with low rates of spontaneous resolution from adolescence onwards. A decreasing SPT wheal size predicted tolerance, while an increasing wheal size predicted persistence (22-24). Spontaneous resolution of early-onset (younger than 18 months) peanut allergy mostly occurred by 6 years of age and occurs much less frequently after 10 years old (25).

What Can We Do about This Rising Trend?

Strategies to counteract the increase in prevalence of peanut allergy can be considered as a pyramid, beginning with primary prevention of those at risk of peanut allergy through earlier introduction of age-appropriate forms of peanut into the infant diet, to secondary prevention of peanut-sensitised children through improvements in the correct diagnosis of peanut allergy and finally the treatment of children with proven peanut allergy (Figure 1).

Primary Prevention

Primary prevention targets children who have yet to develop any manifestation of the disease – which, according to the World Health Organisation definition, would include sensitisation in the absence of clinical reactivity. The Learning Early About Peanut (LEAP) study (15) in the UK demonstrated for the first time that introduction of peanut into the infant diet prior to 12 months of age reduces the risk of developing peanut allergy, in contrast to peanut avoidance until age 5 years. The effect was more marked in children with existing egg allergy or significant eczema, with a relative risk reduction of 70% to 86% when compared to strict peanut avoidance until age 5 years. The benefit of earlier peanut introduction persisted when peanut intake was ceased for 12 months (26). However, the LEAP study excluded children with SPT wheals greater than 5mm at baseline. In addition, whether there is a "window of opportunity" by which peanut should be introduced is controversial. Of note, the intervention did not prevent all peanut allergy: 1.9% of those with negative SPT and 10.6% of those with SPT of 1-4 mm developed peanut allergy despite earlier introduction (15).

The Enquiring About Tolerance (EAT) Study evaluated whether a more pragmatic approach to

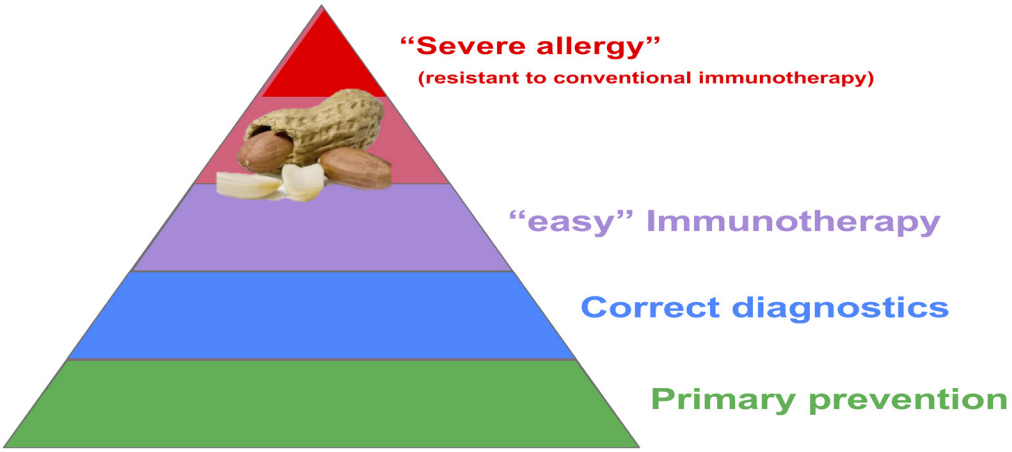

Figure 1. Strategies to counteract the rise in prevalence of peanut allergy. Primary prevention strategies can be applied at a population level to reduce the incidence of peanut allergy, while improved diagnostics can avoid false positive diagnostic tests which might also increase risk of peanut allergy when dietary avoidance is recommended in peanut-sensitised but tolerant children. For those with peanut allergy, immunotherapy can be instituted but there remains around 20-40% of individuals whom experience frequent adverse events with immunotherapy, many of whom will not tolerate the treatment.

early introduction of 6 allergenic foods (peanut, cooked egg, cow's milk, sesame, whitefish, and wheat) in breast-fed infants without specific risk factors for food allergy would protect against the development of food allergy (27). Overall, the study did not find significant reduction in food allergy rates between the early and standard introduction groups by intention-to-treat analysis, however there was a significant reduction in risk by per-protocol analysis. A subsequent secondary analysis reported a significant reduction in food allergy in infants at higher risk of food allergy (for example, those with more severe eczema at enrolment, or with polysensitisation) (28). Many infants in the study struggled to adhere to the earlier introduction of allergens – particularly egg – and where introduction was successful, a lower risk of food allergy was identified, at least for peanut and egg. This highlights the challenges faced when counselling families about primary prevention – adequate intake of foods containing the allergen (which are currently undefined) may only be feasible and achievable by the most determined and motivated families.

Some guidelines advocate for the screening of infants at higher risk of developing food allergy (29), however the benefits of this have not been proven (30). Lack of access to screening may result in a delay in introduction, which could increase the risk of the infant developing a food allergy. Screening is not generally offered in countries such as Israel, where peanut is introduced in infancy, and to date, this has not caused major public health concerns. However, some infants will already be allergic to some of these foods when introduced into the diet (Figure 2). Infants with moderate-severe eczema and/or eczema which began in the first 3 months of life seem to be at greatest risk of reacting to egg and peanut when these are introduced into the diet – but where tolerated, these infants will benefit most from earlier introduction.

Improving Diagnostics

The diagnosis of peanut allergy is best made on the basis of a typical history of IgE-mediated reaction i.e. cutaneous hives, angioedema, gastrointestinal symptoms such as abdominal pain and vomiting, or anaphylaxis involving the cardiorespiratory system. However, diagnosis is often based on the presence of IgE-sensitisation without clinical history – particularly in the current context of 'screening' for potential peanut allergy in very young chil-

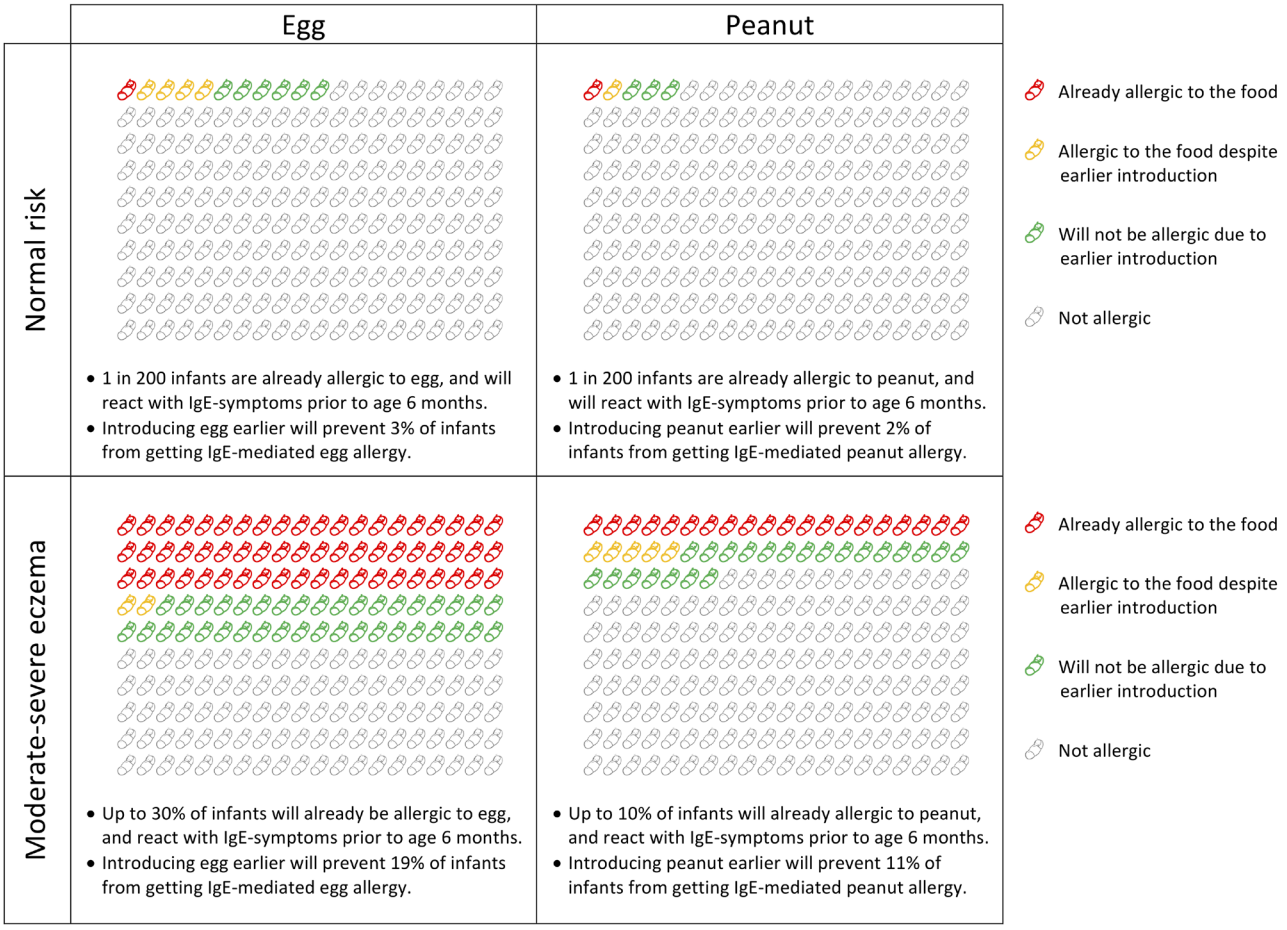

Figure 2. Impact of earlier introduction of egg and peanut into the infant diet before 12 months of age. A proportion of infants (shown in red) will already be allergic to the food when introduced, but to date, no life-threatening reactions have been reported in this context. Those infants shown in green represent those children who will avoid developing food allergy due to earlier introduction. Data from J Allergy Clin Immunol Pract. 2018;6:367-75. doi: 10.1016/j.jaip.2017.12.015. Image reproduced with permission from British Society for Allergy and Clinical Immunology (BSACI).

dren. Unfortunately, IgE-testing – both skin prick testing and serum-specific IgE in the blood, are associated with a high false positive rate of up to 50%, particularly in infants (8). This highlights the need to distinguish correctly between sensitisation – where an individual has peanut-specific IgE (either in the blood or attached to skin-resident mast cells) but does not react to oral exposure and clinical allergy.

The magnitude of the skin prick test wheal size and/or food-specific IgE result does correlate with the *likelihood* of an allergic reaction, but not the *severity* of reaction (31). Clinically useful decision points which are often based on 95% Positive Predictive Values (PPV) have been reported for both tests. For example, a skin prick wheal size to peanut of ≥8mm has been found in most studies to yield a PPV of ≥95% (i.e. there is at least 95% chance that a child with peanut wheal size of ≥8mm will react during a peanut food challenge) (32-35) and high specificity (36). However, these endpoints – particularly for peanut-specific IgE in blood – have not been defined for infants and very young children, despite their use being recommended in guidelines from USA (29). It is for this reason that the oral food challenge remains the gold standard for a food allergy diagnosis, to avoid unnecessary dietary limitations.

Component Resolved Diagnostics

IgE testing to individual components of the peanut protein, rather than then whole protein, has been shown to discriminate better between peanut sensitisation and peanut allergy. In particular, IgE against Ara h 2, a major seed storage protein that is resistant to both heat and digestion, has been shown to have better discrimination when compared to crude peanut-specific IgE (37-39). This is clinically useful, especially when the SPT and/or peanut-specific IgE fall within the equivocal range (≥3mm and/or ≥0.35kU/L, but lower than their respective cut-offs for 95% PPV) (40, 41). The diagnostic utility of other peanut components, in particular Ara h 1, 3, 6 have been less consistent than Ara h 2 (40-42). However, even when using component-resolved diagnostics, there will be 5% of cases where there is a degree of diagnostic uncertainty, and so the need to undertake food challenges to clarify the diagnosis. Furthermore, component testing for peanut allergy only confers a small diagnostic advantage over SPT (39). Despite many advances into the management of peanut allergy, diagnosis and the detection of resolution remains an imperfect science, and clinicians should have a low threshold for undertaking food challenges under safe and appropriate medical supervision to clarify diagnosis.

Active Management

Until recently, the mainstay of management was strict peanut avoidance and the provision of rescue medication for the treatment of accidental allergic reactions. However, even with appropriate dietary avoidance, accidental allergic reactions are common, with 1 in 8 peanut-allergic children experiencing an accidental reaction annually (43).

Food immunotherapy as a form of *active* management for patients with food allergies, has recently generated significant interest amongst all stakeholders. Although the first case of food allergy desensitisation was described in 1908 (44), it has taken over a century for this to evolve into large, multi-centre phase 3 trials. Whether immunotherapy for food allergy is ready for routine clinical practice is still a subject of ongoing heated debate (45, 46). A systematic review and meta-analysis demonstrated that allergen immunotherapy offers a substantial benefit (risk ratio (RR)=0.16, 95%CI 0.10 to 0.26) in raising the threshold of reactivity to the specific food allergen while receiving immunotherapy – desensitisation (47). It is worth noting however that there was significant heterogeneity in the meta-analysis, across different populations, interventions and outcomes**.** Desensitisation outcomes, ranging from achieving a pre-specified clinical threshold or a certain-fold increase in individual threshold at exit food challenge, to a lack of symptoms to a daily maintenance dose, were all grouped together for this meta-analysis.

A more recent meta-analysis specific to peanut oral immunotherapy (OIT) confirmed efficacy of desensitisation (RR in passing exit food challenge = 12·4, 95%CI 6·8 to 22·6) (48). However, the meta-analysis also highlighted the safety concerns: the risk of anaphylaxis in patients undergoing peanut OIT was 3-fold greater in those undergoing OIT, compared to peanut avoidance (RR=3.12, 95%CI 1.76 to 5.55). This estimation of risk is likely to be an underestimate, given the heterogeneity of the reporting of adverse outcomes (46).

Though the risks involved in OIT are not unexpected, trading treatment-related allergic reactions at home or in hospital (arguably a more controlled setting) for unpredictable, accidental exposures in the community appears to be a risk many patients and their families are willing to take (47). This may well result from a 'resetting' of the expectation of a severe reaction that occurs during OIT: the experience of controlled reactions under medical supervision itself has a significant impact on health-related quality of life (HRQL) measures, and one group has reported that around one third of the overall improvement in HRQL with OIT is linked to the screening challenge that is usually undertaken prior to starting treatment (49). As yet, there is a paucity of efficacy and safety data relating to longer term follow-up, which needs to be urgently addressed.

Alternatives to OIT exist. Epicutaneous immunotherapy (EPIT) to peanut is a novel approach which involves transdermal administration of peanut allergen using a patch device with the objective to induce tolerance. The recently completed clinical trials for the peanut EPIT device has shown excellent safety profile with low withdrawal rates, albeit only a modest increase in cumulative reactive dose after 12 months of treatment (50). Longer treatment durations may have greater efficacy, but this needs to be confirmed.

Sublingual immunotherapy (SLIT) is a well-studied method of immunotherapy in individuals with allergic rhinitis. There are far fewer trials of SLIT to peanut (51-53), with only one head-to-head comparison of SLIT to OIT (54). Given the log-fold lower treatment dose in SLIT compared to OIT, it is not surprising to find that SLIT is able to induce modest levels of desensitisation (SLIT patients reaching lower eliciting dose thresholds than OIT), but with an excellent safety profile – adverse events mainly involving oropharyngeal symptoms usually not requiring treatment, with rare systemic symptoms or need for adrenaline (55). Longer durations of treatment appear to be associated with a greater treatment effect (52).

Irrespective of route of administration, the desensitisation is, in most cases, temporary. The majority of the published data relates to OIT: when peanut-OIT is stopped for 4–6 weeks, over half of patients lose their levels of desensitisation (46) i.e. patients do not demonstrate tolerance (prolonged immune unresponsiveness that persists after withdrawal of the allergen). This loss of desensitisation appears to increase where avoidance occurs for a longer duration post treatment (56). Given the lack of data in this area, the term sustained unresponsiveness has been introduced as an outcome in immunotherapy trials to describe the state of unresponsiveness after a period of allergen avoidance following food immunotherapy. Sustained unresponsiveness is more likely in patients with initial lower levels of sensitisation (56), but baseline markers of sensitisation do not currently predict sustained unresponsiveness in a clinically-predictive manner. Longer term efficacy is clearly important to patients; the lack of data to inform longer-term efficacy (beyond 1 year of treatment) and safety is a major gap in evaluating OIT for clinical practice.

Eosinophilic oesophagitis (EoE) is a chronic, immune-mediated disease characterized by eosinophil infiltration of the oesophagus. Symptoms of EoE include abdominal pain, vomiting, reflux, anorexia, dysphagia, food impaction and chest pain. There is evidence suggesting an increased risk of EoE in patients undergoing OIT/ SLIT (57). As most trials do not routinely use endoscopy to confirm the diagnosis of EoE in suspected cases, estimates for the rate of EoE in patients undergoing OIT range from 5.1% based on biopsy-proven cases to as high as 34% based on symptoms only (58).

The role of adjuvants with food immunotherapy has been studied in several small trials, with the hope of improving on the two current major drawbacks of conventional immunotherapy – high rates of adverse events and poor efficacy in sustained unresponsiveness. Adjuvants such as anti–immunoglobulin E monoclonal antibody (omalizumab) and probiotics have shown some promise in mitigating the above two issues respectively (59). Omalizumab used as an adjunct to OIT seems to be effective in reducing reactions during OIT dose escalation but reactions may resume once omalizumab is discontinued. Omalizumab added to OIT has not been found to increase the likelihood of OIT-induced desensitisation or sustained unresponsiveness (59). One study evaluated peanut OIT with co-administration of a probiotic (Lactobacillus rhamnosus); the authors report high rates of desensitisation (90%) and notably high rates of sustained unresponsiveness (82.1%), however the latter was assessed at varying timepoints, from as little as just 2 weeks off OIT (60). This, combined with a lack of a intervention arm in which participants received OIT without the probiotic unfortunately limit both comparisons to conventional peanut OIT, and the interpretation of efficacy and longer-term outcome data (60, 61).

Finally, at the top of the pyramid are those with "severe peanut allergy" – patients with a history of severe or refractory anaphylaxis to peanut (and

whom are considered at too high risk for conventional OIT), or those who have failed peanut OIT due to the frequency or severity of adverse reactions. In general, about 20% of patients undergoing OIT fall into this category (62). It is possible that SLIT or EPIT may be a more pragmatic option for these patients, perhaps allowing them to then undergo OIT at a later stage, but this group of patients have to date been excluded from clinical trials (62). There remains a need to provide better education for both patients/their families but also the food industry, to improve the awareness of food allergies, and the optimal recognition and management of reactions.

In the meantime, clear and transparent information about the risks and benefits of allergen immunotherapy must be communicated to patients and caregivers; we would contend that allergen immunotherapy should only be carried out in centres with experience in managing anaphylaxis and undertaking desensitisation.

Future Directions

With the widespread change in allergy prevention guidelines across the world following findings from the LEAP study (15), the paradigm in managing peanut allergy has changed. Pragmatic clinical studies are needed to explore the longer-term feasibility and cost-effectiveness of primary prevention in both high risk infants and the wider population. For those with peanut allergy, more data are needed to improve safety and longer-term outcomes (such as sustained unresponsiveness), before allergen immunotherapy can be considered the standard of care for peanut-allergic children.

Conclusion

The management of peanut allergy in children is gradually shifting from one of passive allergen avoidance and treatment of reactions to one of prevention, active desensitisation and tolerance induction. With this shift, the diagnosis of peanut allergy should no longer be seen as a life sentence. More data is needed to improve the new treatment strategies available, so that we are able to offer personalised immunotherapy to optimise safety and longer term efficacy.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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Historical Article

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Jadwiga Olszewska: A Woman Pioneer in Medicine in Serbia and Bosnia and Herzegovina

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Abstract
This article describes the life and medical activities of Jadwiga Olszewska (1855–1932) in Serbia from 1895–1899, Austro-Hungarian (AH)-occupied and annexed Bosnia and Herzegovina (BH) from 1899–1918, and the newly founded Kingdom of Yugoslavia, from 1919–1932. In summer 1899, Olszewska replaced Teodora Krajewska as an AH official female physician in Tuzla. Born in Congress Poland, Olszewska had enrolled in 1873 in the medical courses for women in St. Petersburg but had left Russia in 1880 to study medicine in France. She had lived as a student and single parent in Paris since 1883, and she was awarded her Doctorate in Medicine from the University of Paris in 1894. She could not practice medicine in Russian-occupied Poland because of her French diploma, and she could not practice in most Western countries due to her gender. Therefore, she decided to move to Serbia, where she worked as an assistant physician in the district hospitals of Loznica (1895–1897?) and Požarevac (1897–1899). Driven by the need for a higher income to fund her son's education, she engaged her network of Polish compatriots and procured the position of an AH official female physician of Tuzla in 1899, where she performed her duties in an exemplary manner. After the breakdown of the Austro-Hungarian Empire (AHE) in 1918, Olszewska remained in Tuzla and retired as a Yugoslav official physician in 1923. When she died in Tuzla in 1932, local colleagues had to arrange for a proper funeral because Olszewska did not leave any savings due to her insufficient pension. Olszewska's grave never received a tombstone, and it is untraceable today. **Conclusion.** Jadwiga Olszewska (1855–1932) was a woman pioneer of medicine from Poland, who practiced her profession first as an assistant physician in Serbia (1895–1899) and then as an AH and Yugoslavian official female doctor in Tuzla, BH (1899–1923).

Key Words: Official Female Doctors ▪ Jadwiga Olszewska ▪ Tuzla ▪ Bosnia and Herzegovina.

Introduction

Jadwiga Olszewska is exceptional as a first-generation female pioneer of medicine in Serbia and Bosnia and Herzegovina (BH). Born in Poland, she practiced her profession in the few areas that were available to female physicians in the 19th century. Nevertheless, no exhaustive biography of her person has been provided by women's historians or Polish and former Yugoslavian historians and biographers. Overlooked in the history of women medical pioneers, Olszewska is only briefly mentioned in the context of Serbian, Yugoslavian, and Bosnian national and local histories of medicine and public health (1-6). Polish contemporaneous and recent sources on Polish emigrés and their networks and communities include Olszewska (7, 8, 9) but only as the protegée of her more prominent compatriot and colleague Teodora Krajewska, the Austro-Hungarian (AH) official female doctor Olszewska replaced in Tuzla. This neglect is likely due to the fact that Krajewska's memoirs (10) are the only detailed source of information about Olszewska.

Krajewska explains that after she had been invited to continue her service in Sarajevo in 1898, it was important to her to choose a suitable successor for her practice in Tuzla (10). A Polish compa-

triot brought Jadwiga Olszewska to her attention because Olszewska was a Polish female medical doctor who was working in the closely situated Serbian city of Loznica and would likely be interested.[1] After further investigation, Krajewska decided to encourage Olszewska to apply for the advertised position in Tuzla.[2] She knew that the AH authorities were unlike to employ "Russians" because of suspected "nihilism", and she therefore intervened for Olszewska with de facto governor Benjamin de Kállay in Vienna (10). Olszewska was appointed the provisory official female doctor of the district of Tuzla in May 1899, and her first name was Germanised as "Hedwig". Olszewska arrived in BH to receive training from her predecessor several weeks prior to the arrival of Gisela Januszewska, the simultaneously appointed official female physician of Banjaluka.[3] She was the fourth of nine AH official female physicians who came to BH to staff the institution of AH "Amtsärztinnen" (official female physicians) for Bosnian Muslim women, which was created in 1891.

In the 1980s, Ctibor Nečas (1991), historiographer of the AH institution of official female physicians in Bosnia and Herzegovina (BH) (12), began to search for more information about Jadwiga Olszewska (13). His biographical sketch, published in 1987, revealed Olszewska's journalistic and literary work for Polish newspapers and magazines, her short marriage in 1882, and her status as a single parent who was constantly under financial pressure. Nečas also determined the correct date and circumstances of her death in Tuzla in 1932 (13). Nečas found evidence that Olszewska had worked as a hospital doctor in Požarevac in Serbia and incorrectly concluded that she had not worked in Loznica as Polish sources had indicated (13). A recent investigation in Serbian archives by Husref Tahirovic revealed that Olszewska was employed as an assistant physician in Loznica in 1895 (14) and moved to Požarevac (15) to work in the same function in 1897 (5) before she was appointed an AH official female doctor in Tuzla in 1899.[4]

Jadwiga Olszewska's life and medical activities, first in Serbia and then as an AH official female physician in BH, are the subject of this review. The review is based on Nečas' biography and Krajewska's memoirs, as well as new archival materials and various Yugoslav, Serbian, and Bosnian sources.

Olszewska's Short Biography

Jadwiga Olszewska was born on April 10, 1855 in Kuzawka (16) near Slawatycz. Today a village in Eastern Poland next to the Russian border, Slawatycz was then situated in Russian-occupied Poland. Nothing is known about Olszewska's family background, except that her mother was a teacher (17). Olszewska attended a girls' gymnasium in Warsaw and took her school leaving examination in 1873. Subsequently, she moved to St. Petersburg, where medical courses exclusively for women had been established at the Military Medical Surgery Academy in 1872.[5] She enrolled in a four-year programme for "learned obstetricians" ("uchenye akusherki"), a qualification that entitled female graduates to practice as physicians without being awarded a doctorate (18). Olszewska did not finish the programme; instead, she left Russia when the Tsarist persecution of radical students reached an initial climax. Many female students from Russia and Russian-occupied Poland left at this time to

[1]By 1898, Olszewska had already left Loznica to work in Požarevac.

[2]The advertised position designated Tuzla as the site of operation (11), but apparently there was no advertisement for the newly created position in Banjaluka, which Gisela Januszewska filled in 1899.

[3]Cf. Gisela Januszewska (née Rosenfeld), an Austro-Hungarian 'Woman Doctor for Women' in Banjaluka, 1899–1912. In: AMA 49/1 (2020): 75-83.

[4]Unfortunately, this research did not uncover a portrait photograph of Jadwiga Olszewska, but see Picture 6 (Jadwiga Olszewska in the company of friends in Sarajevo in 1905).

[5]For more information regarding the situation of women studying in the Russian Empire under diverse changing discriminations, see e.g., Barbara A. Engel. "Women Medical Students in Russia, 1872-1882: Reformers or Rebels?", Journal of Social History 12, 1979: 394-415; Ruth A. Dudgeon. "The Forgotten Minority: Women Students in Imperial Russia," Russian History/Histoire Russe 9/1 (1982): 1-26; Johanson, Christine. Women's struggle for higher education in Russia, 1855-1900. Kingston: McGill-Queen's University Press,1987; Koblitz, Ann H.: Science, Women and Revolution *in Russia*. Women in Science. Amsterdam et al.: Harwood Academic Publishers, 2000.

pursue studies in Switzerland and France.[6] In 1880, Olszewska settled in Paris, a site of refuge for many Polish émigrés from the late 18th century through the 19th and early 20th centuries. In 1881, Olszewska enrolled in the Medical Faculty of the University of Paris (17).[7] She interrupted her studies to marry Bartlomiej Boldireff in 1882. She separated from Boldireff before the birth of her son Włodzimierz on January 15, 1883 (13, 19). As a young mother, she continued to pursue her medical studies under her own name "Olschewska" (13).

Włodzimierz Boldireff-Streminski (1883-1970)[8] was a reputed civil engineer in Poland in the interwar years and was also a noted pioneer of Tatry mountaineering (Picture 1). Olszewska relied on her Polish network to help her raise her son and generate an income. In the 1880s, she began to write for Polish newspapers and magazines in Cracow and Lviv, and she continued to pursue journalistic and literary activities as an assistant physician in Serbia and an official female physician in BH (13). In her journalistic work, Olszewska advocated for women's equal rights and criticized educational and professional discrimination against women (20). She was committed to the cause of higher education for girls and was among the most avid donors at charity events for the Serbian "Belgrade Women's Association", which raised funds to support this cause (21).

Picture 1. Włodzimierz Boldireff-Strzemiński, Jadwiga Olszewska`s son. A detail of the Picture "Sekcja Turystyczna Polskiego Towarzystwa Tatrzańskiego na Hali Gąsiennicowej, 1910. With permission of Museum sport and tourism in Warsaw.

In 1894, Olszewska defended her thesis regarding the clinical study of typhoid-related myocardial disorders among children (22) (Picture 2). She was awarded her medical doctorate from the Medical Faculty of the University of Paris at the age of 39 (17).

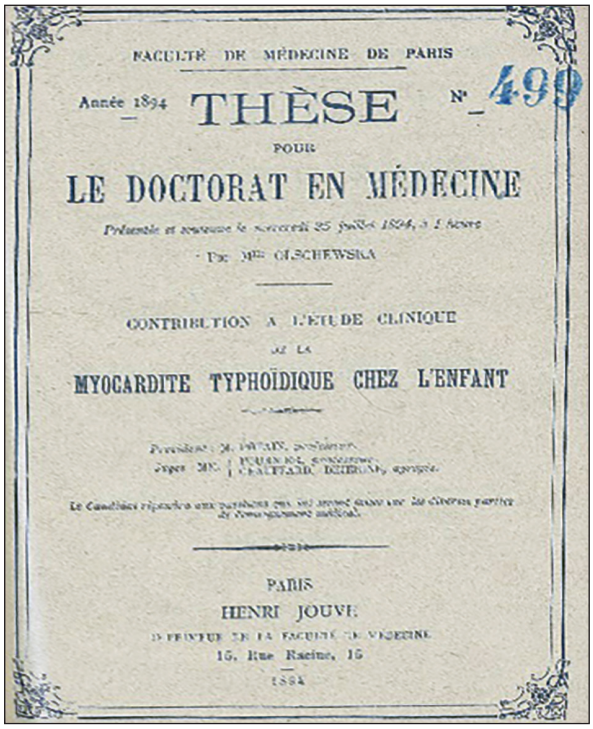
Olszewska had planned to return to Poland with her son upon completion of her studies; however, the Russian authorities were not recognising foreign diplomas (18). The only alternative for Olszewska was to seek employment in the Kingdom of Serbia, where female physicians could obtain permission to practice as assistant physicians in hospitals.[9] On April 28, 1895, she wrote a letter of

[6]The overwhelming majority of female students graduating from Swiss and French universities in the late 19th and early 20th centuries were from the Russian Empire (including Congress Poland), see Daniela Neumann: Studentinnen aus dem Russischen Reich in der Schweiz (1867-1914), Zurich: Rohr, 1987; Sigrist, Natalia Tikhonov. Les femmes et l'université en France, 1860-1914. Pour une historiographie comparée. In: Histoire de l'éducation 122/2009, pp. 53-70, p. 57; see also Lipinska (9) and Schultze C. Women physicians in the 19th century [in French]. Paris: Ollier-Henry; 1888. p. 16.

[7]French universities were closed to (French) women prior to the 1860s because they were excluded from higher education. Female emigrants from the Russian Empire were not subjected to the required higher education entrance qualification; cf. Christen-Lécuyer Christen-Lécuyer, Carole. Les premières étudiantes de l'université de Paris. In: Travail, genre et sociétés (Dossier: Histoire de Pionnières), 2000/2, no. 4, pp. 35-50; Pigeard-Micault N. "Nature féminin" et doctoresses (1868-1930). Histoire, médecine et santé. 2013;3:83-100. https://doi.org/10.4000/hms.

[8]Włodzimierz Boldireff assumed the name "Streminski" (the name of his wife's grandfather) in 1925 to Polonize his Russian name.

[9]In the Kingdom of Serbia (1882–1918 according to the Julian calendar), all doctors in the civil and military service were


FACULTÉ DE MÉDECINE DE PARIS

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POUR

LE DOCTORAT EN MÉDECINE

Présentée et soutenue le samedi 25 juillet 1894, à 1 heure

Par Mlle OLSCHEWSKA

CONTRIBUTION A L'ÉTUDE CLINIQUE

DE LA

MYOCARDITE TYPHOÏDIQUE CHEZ L'ENFANT

Président : M. [illegible]

Juges : MM. [illegible]

Le Candidat répondra aux questions qui lui seront faites sur les divers points de l'enseignement médical.

PARIS

HENRI JOUVE

IMPRIMEUR DE LA FACULTÉ DE MÉDECINE

15, Rue Racine, 15

1894

Picture 2. Front page of "Mlle Olszewska's" thesis "Contribution à l`étude clinique typhoidique chez l'enfant", Paris, 1894. With permission BIU Santé (Paris).

inquiry to the Serbian Ministry of the Interior in Belgrade to offer her service as an assistant doctor at "any district hospital near the railway" (Picture 3). On May 6, the Ministry employed Jadwiga Olszewska as an assistant physician in the Hospital of the District of Podrinje, situated in the district capital Loznica (Picture 4), a small city in Western Serbia near the Bosnian border.[10] She left her son in the care of her compatriot and friend Bronislawa (Bronia) Sklodowska-Dluski (1865-1939)[11] in Paris and departed for Serbia.

Sources related to Olszewska's membership in the Loznica branch of the "Belgrade Women's Society" (BWS) (23) indicate that she had joined the society immediately upon her arrival in Loznica. In early 1896, she asked the BWS for a loan and designated the local merchant Svetolik Popović as her guarantor (24); she obviously lacked a sufficient income to support her son. Indeed, the need to improve her financial situation was a dominant motive of Olszewska's life from the beginning of her professional career. In the same year, Olszewska sought transfer to a location where she would be able to open a private practice. She was transferred to the District Hospital of the District of Požarevac[12] (Picture 5), a city near Belgrade in Eastern Serbia; however, her plans to practice as a private doctor did not come to fruition (10).

As an assistant physician in Požarevac, Olszewska applied to the official female physician position in BH advertised in 1897 (11). However, she was quickly informed that they had not "drawn her into consideration" (12). Rather than giving up, Olszewska put her Polish network into motion, and in 1898, Krajewska chose her as a successor for her practice in Tuzla and intervened for her in Vienna. The responsible authorities assessed Olszewska's application and accepted it in March 1899, citing her pre-existing local language skills, (10), and Olszewska travelled to Cracow to acquire Austrian citizenship. Soon after, she travelled to Tuzla to meet Krajewska, who had learned of her own transfer to Sarajevo and the obligation to instruct her successor (10).

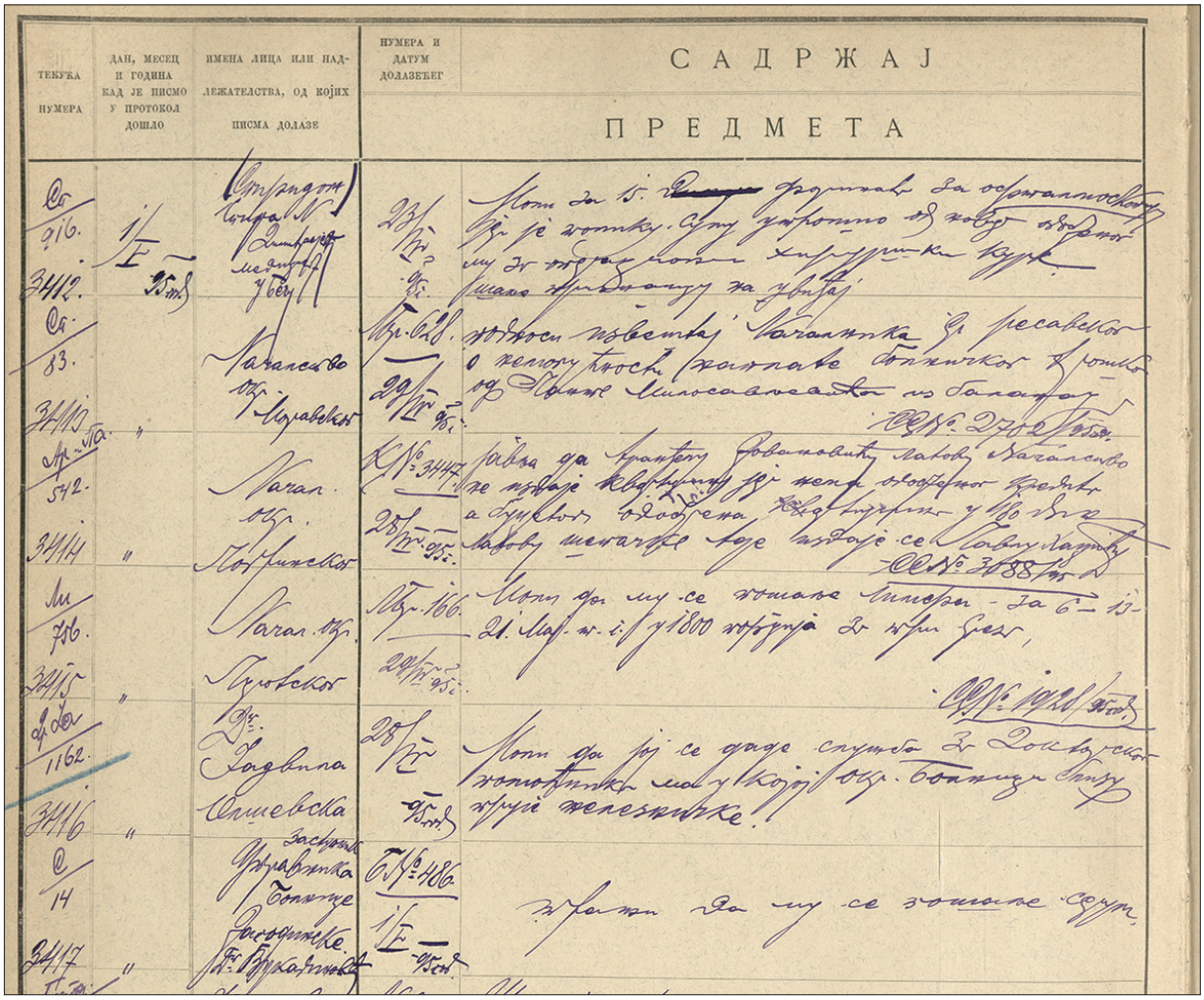
From the last week of May 1899, Krajewska and Olszewska worked together in Tuzla and apparently separated as good friends. Krajewska described Olszewska as a pleasant woman who had a taste for the bohème and was "awfully dressed" (10). Bronislava Prašek Calczynska (1887-1969),

appointed by decree of the ruler, though a woman doctor could not be appointed by decree. Medical assistants were appointed by prescription of the Minister of the Interior.

[10]Working protocol of the Sanitary Department of the Ministry of the Interior of Kingdom of Serbia for 1895, SN0 3416.

[11]According to Boldireff-Streminski, who lived in Paris until 1898 under the care of Marie Sklodowska-Curie's elder sister Bronislawa (Bronia) Sklodowska-Dluski (1865-1939), who practiced as a gynaecologist in Paris and maintained a "salon" as a meeting point for exiled Poles.

[12]The archival sources do not allow to reconstruct the exact point in time when Olszewska's moved to Požarevac, although the record of her membership at the local branch of the "Belgrade Women's Society" (BWS) provides clues as to the time and reason for the transfer (Milanović J, Jovanović Simić J. Female physicians and doctor's wives - members of the Women's Society (1875–1915) in Serbia). Srp Arh Celok Lek. 2020: DOI: https://doi.org/10.2298/SARH191106078M).



Picture 3. Archives of Serbia. Page of the Protocol of 1895 of the Ministry of the Interior - Health Care Department which mentions Dr. Jadwiga Olszewska's application sent on 28th April 1895 (No. 3416). Published with kind permission of the Archives of Serbia.

Picture 4. Loznica at the time when Dr. Jadwiga Olszewska started working there. Published with kind permission of Museum of Jadar, Loznica.

Picture 5. Požarevac at the time when Dr. Jadwiga Olszewska started working there. With Permission from the Historical Archives of Požarevac. (The Pozarevac District Town Hall, built between 1888 and 1889. Historical Archives of Pozarevac/ Republic of Serbia, Digital Collection of Tanasije Bata Dimitrijevic, sign. 044a.)

Picture 6. Jadwiga Olszewska „sitting in the first row with both hands raised up" in the company of friends in Sarajevo around 1905. By courtesy of the Department of the Old Medical Book of the Central Medical Library in Warsaw. Reference Number GBL-I-1327 (103).

Jadwiga Olszewska died on February 28, 1932 in Tuzla (1, 25). Her small pension, which had not sufficed to cover her cost of living, did not cover the expenses of a funeral either. Compassionate colleagues collected money to organise a funeral at the Roman Catholic cemetery of Tuzla. No tombstone was placed, and the location of Olszewska's grave has since vanished, along with the wooden cross that showed her name (5).

who met Olszewska in 1915, confirmed this impression, depicting her as a winning companion but strikingly unattractive and neglectfully dressed (Picture 6) (4). Olszewska was reputed to be a woman of high literary education who spoke numerous languages fluently (16).

Olszewska was appointed as an AH official female physician on May 24, 1899. She took the oath of office upon her arrival in Tuzla, where she was expected to "provide medical help to all women in the city and its surroundings, regardless of their social status, nationality or religion, with special attention to Muslim women" (12). Krajewska (10) noted that Olszewska seemed happy with her new position, particularly for financial reasons, though her financial situation continued to be difficult. Olszewska was at the forefront of the AH official female physicians' effort to win equal pay for female and male AH official doctors and an increase of their basic salary (12). Her desperate financial situation likely also influenced her decision to stay in BH after the AHE collapsed in 1918. Olszewska took an oath of allegiance to the Kingdom of Yugoslavia on March 1, 1919 (Picture 7), and she remained in her position as an official physician in Tuzla until her retirement in 1923 at age 68.

ZAKLETVA.

Ja Dr Jadwiga Olszewska zaklinjem se svemogućim Bogom, da ću vladajućem kralju PETRU I. vjeran biti, da ću se Ustava savjesno pridržavati i da ću dužnost moju po zakonima i zakonskim naredbama predpostavljenih mi vlasti tačno i savjesno otpravljati.

Potpis:

Dr Jadwiga Olszewska, ovlašćena ljekarica

Potvrdujem, da se je Dr Hedwiga Olszewska ovlašćena ljekarica danas predamnom zakleo.

1. Marta 1919
(Datum).

Potpis:

Zastupnik nadležnika odnosa.

Svjedok:

Ahmet Ahmetović
pos. oficijal

53207/19

P. br. 9618. 1918/19.

Picture 7. Jadwiga Olszewska's Oath of Allegiance to King Peter the First stating that she will abide by the constitution and exercise her duty according to the laws and regulations. Archives of Bosnia and Herzegovina.

Olszewska's Professional Activities

A recent search in Serbian archives revealed that Jadwiga Olszewska began her professional career as an assistant physician in the city hospital of Loznica in 1895 (26) and worked in the same function in the city hospital of Požarevac in 1897 (27). The sources do not specify the exact date of her transfer to Požarevac, but the record of her membership in the Loznica branch of BWS indicates that she moved to Požarevac in late 1896 or early 1897 because she was not on the list of members for the second half of 1897 (28).

Jadwiga Olszewska's activities as an official female physician in Tuzla and Tuzla district have been documented in detail in Nečas' collective biography of the AH official female physicians in occupied BH, published in 1992 (12). Tuzla, referred to as "Dolnja (Lower) Tuzla" in AH sources (Picture 8), was known for salt production and was considered an emerging industrial town in the late 19th century (29). At that point, the town had about 10,200 inhabitants, and about 60% of the inhabitants were Muslim.

When Olszewska arrived in Tuzla in May 1899, Krajewska introduced her to the local clientele, including the Muslim ladies of the city (10). They worked together in Krajewska's practice and travelled together on an official trip to the rural subdistricts of Modrica and Gracanica. When Krajewska left Tuzla at the end of June, Olszewska was sworn into her office and began service as a provisory official on June 21, 1899 (16). She gained permanent status in June 1901 (12). Olszewska had an advantage in that the local outpatient clinics for women and children – which had been established by Anna Bayerová in 1892 (30) and taken over by Krajewska in 1893 – were already familiar to the local population.

According to official statistics, Olszewska treated 395 (mostly female) patients from July to December 1899 and an average of 850 patients annually in the following years (31). These figures included patients from the city who visited her in her practice and those whom she visited on official trips to various cities and rural sites of the district. Like all official female physicians in BH, Olszewska functioned as a general practitioner and was most frequently consulted for "diseases of the digestive system" and "diseases of the genital organs", followed by respiratory and skin diseases and metabolic disorders, including rheumatic and osteomalacic affliction as well as rickets. Many of her patients suffered from poverty-related chronic infectious disorders such as tuberculosis, scrophulosis and endemic syphilis (32). About 25% of her patients were children; notably, the average proportion of child patients among official female doctors was about one-third (31). The proportion of Muslim patients in the practice had increased rapidly, from 37% in 1893 to 64% in 1897, due to Krajewska's zeal for "approaching" local Muslim women. However, this trend reversed itself under Olszewska's leadership: 64% of her patients were Muslim in 1899, and the proportion fell to 46% in 1900 and 37% in 1901 (31).

Picture 8. Tuzla at the time when Dr. Jadwiga Olszewska started working there at the end of the 19th century. From "Tuzla in Memory" by Enver Mandžić. With permission of the author.

Among the most important tasks of the official female physi-

cians was to participate in the eradication of syphilis, a public health priority of the AH authorities in BH since 1890 (33). Based on the expert opinion of Isidor Neumann, professor of Dermatology and Venereology at the University of Vienna, the authorities in BH had refrained from the common method of hospitalizing rural syphilitics by use of military force (34). Rather, official physicians were instructed to treat infected locals on-site. Female physicians were therefore required to examine and monitor the patients, particularly the rural female Muslim population (33). In 1900, Olszewska became involved in a local syphilis eradication in Maglaj, which was one of several rural foci of endemic syphilis in her district. She was ordered to treat the 168 infected locals (of both sexes) with an iodine tincture for several years and expected to monitor the patients' adherence to the prescriptions (12).

Olszewska's duties as an official physician included the control of infectious diseases, such as carrying out vaccinations on a regular basis throughout the district and acting as an "epidemics physician" when required. For example, she was involved in combating a smallpox epidemic that had spread from Derventa in Northern Bosnia at the border to Croatia in 1899. She was ordered to Bijeljina and tasked with locating infected women in Muslim households, which were suspected of not reporting infected female members to the authorities. In 1899, Olszewska vaccinated 872 women and children in the subdistricts of Bijeljina and Tuzla (12). According to Prašek Calczynska, she was also actively involved in combating a cholera epidemic in the same region (Derventa) in April 1915 (4).

Concluding Remarks

Jadwiga (Hedwig) Olszewska was a Polish woman pioneer in medicine. She began her study of medicine in St. Petersburg in 1873. Like many Poles and many female students at the time, she left the Russian Empire and settled in France in 1880. She studied medicine in Paris but interrupted her studies for her short marriage in 1882. As a young mother and single parent reliant on emigrated compatriots' support, she resumed her medical studies in Paris and finished in 1894. Despite her plans, she could not return to Congress Poland as a physician at that time, nor did she have the means to open a private practice in France or elsewhere. Among the few options left to her was to work as a hospital doctor in Serbia. Olszewska made considerable and eventually successful efforts to serve as an official female doctor within the AH administration of BH. From July 1899 to 1918, she performed her official duties by working as a general practitioner for women and children in Tuzla and implementing epidemic control throughout the district. She remained on duty as a Yugoslav official physician in 1919 and retired in 1923. Her memory had already vanished by the 1920s, when she still lived in Tuzla as a retired civil servant with an insufficient pension. Olszewska died on February 28, 1932 in Tuzla, where she is buried in an unknown grave in the Roman Catholic cemetery. The renowned physician Sulejman Azabagić (1915-1981) from Tuzla remembered Jadwiga Olszewska as a modest and quiet person who was devoted to her work (5).

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Conflict of Interest: The authors declare that they have no conflict of interest.

Please see the supplementary material entitled: Statistical Data for the Annual Report by the Official Female Physician Dr. Jadwiga Olszewska in Tuzla in 1905.

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Supplementary material

Statistical Data for the Annual Report by the Official Female Physician Dr. Jadwiga Olszewska in Tuzla in 1905

The statistical section of the 1905 official annual report of Dr. Jadwiga Olszewska provides information on her monthly activities and the places in the Tuzla district where she worked (Picture 9). The report provides a monthly presentation of the number of pathological conditions in each year, which is of particular importance because it lists the diseases for which patients sought medical attention. It is interesting to note that Dr. Olszewska examined only 27 patients with acute infectious diseases, a significantly smaller number than the 377 patients with chronic infectious diseases in that year. These data indicate that chronic diseases were a serious health problem for women in Bosnia that forced them to seek medical help, but the same was not true for acute infectious diseases. In Bosnia at that time, the most common chronic diseases were syphilis and tuberculosis, whose symptomatology impaired patients' quality of life. It is also important to note that Olszewska only provided obstetric assistance in seven labours. These data indicate that she was a female doctor who treated all women's diseases. She was not a gynaecologist, as some authors from the former Yugoslavia state in their papers.[13] In fact, Olszewska was primarily a family physician for all diseases of the female population.

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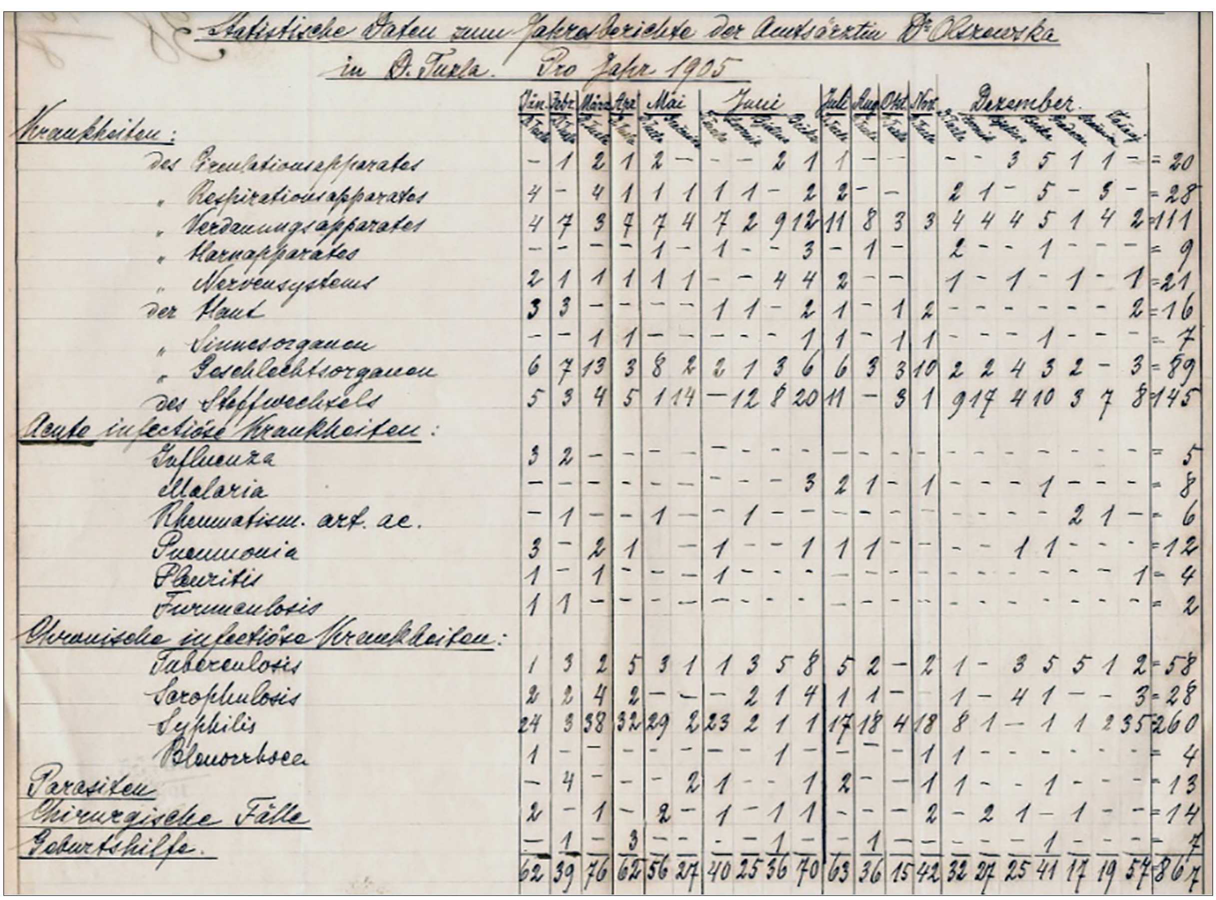

Statistische Daten zum Jahresberichte der Amtsärztin Dr. Olszewska in D. Tuzla. Pro Jahr 1905

Krankheiten:	Jän	Febr	März	Apr	Mai		Juni				Juli	Aug	Okt	Nov	Dezember							
des Zirkulationsapparates	–	1	2	1	2	–	–	–	2	1	1	–	–		–	–	3	5	1	1	–	= 20
„ Respirationsapparates	4	–	4	1	1	1	1	1	–	2	2	–	–		2	1	–	5	–	3	–	= 28
„ Verdauungsapparates	4	7	3	7	7	4	7	2	9	12	11	8	3	3	4	4	4	5	1	4	2	= 111
„ Harnapparates	–	–	–	–	1	–	1	–	–	3	–	1	–		2	–	–	1	–	–	–	= 9
„ Nervensystems	2	1	1	1	1	1	–	–	4	4	2	–	–		1	–	1	–	1	–	1	= 21
der Haut	3	3	–	–	–	–	1	1	–	2	1	–	1	2	–	–	–	–	–	–	2	= 16
„ Sinnesorganen	–	–	1	1	–	–	–	–	–	1	1	–	1	1	–	–	–	1	–	–	–	= 7
„ Geschlechtsorganen	6	7	13	3	8	2	2	1	3	6	6	3	3	10	2	2	4	3	2	–	3	= 89
des Stoffwechsels	5	3	4	5	1	14	–	12	8	20	11	–	3	1	9	17	4	10	3	7	8	= 145
Acute infectiöse Krankheiten:																						
Influenza	3	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	= 5
Malaria	–	–	–	–	–	–	–	–	–	3	2	1	–	1	–	–	–	1	–	–	–	= 8
Rheumatism. art. ac.	–	1	–	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	2	1	–	= 6
Pneumonia	3	–	2	1		–	1	–	–	1	1	1	–	–	–	–	1	1	–	–	–	= 12
Pleuritis	1	–	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	= 4
Furunculosis	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	= 2
Chronische infectiöse Krankheiten:																						
Tuberculosis	1	3	2	5	3	1	1	3	5	8	5	2	–	2	1	–	3	5	5	1	2	= 58
Scrophulosis	2	2	4	2	–	–	–	2	1	4	1	1	–		1	–	4	1	–	–	3	= 28
Syphilis	24	3	38	32	29	2	23	2	1	1	17	18	4	18	8	1	–	1	1	2	35	= 260
Blenorrhoea	1	–	–	–	–	–	–	–	1	–	–	–	–	1	1	–	–	–	–	–	–	= 4
Parasiten	–	4	–	–	–	2	1	–	–	1	2	–	–	1	1	–	–	1	–	–	–	= 13
Chirurgische Fälle	2	–	1	–	2	–	1	–	1	1	–	–	–	2	–	2	1	–	1	–	–	= 14
Geburtshilfe	–	1	–	3	–	–	–	–	1	–	–	1	–	–	–	–	–	1	–	–	–	= 7
	62	39	76	62	56	27	40	25	36	70	63	36	15	42	32	27	25	41	17	19	57	867

Picture 9. Olszewska's Annual Report from Tuzla in 1905. Archives of Bosnia and Herzegovina.

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