

asthma defined by the degree of T_H2 inflammation¹⁰. These data strengthen the argument that epithelial function affects the degree of T_H2 inflammation.

In parallel, the clinical heterogeneity of asthma has been emphasized by unbiased statistical cluster analysis techniques. Moore *et al.*⁴ have described five distinct severe asthma phenotypes that differ in lung function, age of asthma onset, disease duration, atopy, gender, symptoms, medication use and health care usage. The existence of these distinct phenotypes suggests that several pathologic mechanisms promote the variety of symptoms. It would now be useful to analyze people with each phenotype with respect to their epithelial and immune markers to determine whether a molecular signature can be assigned to each clinical phenotype and thus provide clear direction for phenotype specific therapy.

Results from recent clinical trials for a blocking antibody against the T_H2 cytokine IL-5 support the presence of distinct asthma phenotypes. An effect was seen only in subjects with severe asthma resistant to standard treatments, requiring oral steroids and characterized by large numbers of eosinophils^{2,3}. It

is likely that other T_H2 pathways being investigated in asthma as therapeutic targets will only be of benefit in such subjects. Those with low T_H2 responses, and with high numbers of other inflammatory cells, such as neutrophils, who represent at least a third of all severe asthmatics, have a relatively poor response to steroids and thus highlight a clear unmet clinical need.

Current preclinical models have focused almost exclusively on the role of T_H2 -derived inflammation and do not adequately characterize asthma that is not driven by T_H2 responses. Developing new models is not straightforward, given that the mechanisms underlying the characteristic symptoms of non- T_H2 asthma are not well understood. But research points in the direction of factors associated with the innate immune system, such as environmental exposure to bacterial endotoxin, air pollution, ozone and infection history¹¹.

The growing body of genetic and clinical data highlight the need to consider the role of structural components of the airway in the onset and propagation of asthma. In particular, the epithelium may be central, as this surface is the first contact for allergens within the lung.

The environment of allergen challenge must be taken equally into consideration. To reflect the described clinical phenotypes, new models must also take into account age, obesity and gender differences, to enable investigation of how these factors affect epithelial immune interactions after allergen exposure. The task now is to refine or redesign preclinical model systems to enable more effective translation of basic science findings into the clinic.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

1. Koppelman, G.H. *et al.* *Am. J. Respir. Crit. Care Med.* **180**, 929–935 (2009).
2. Nair, P. *et al.* *N. Engl. J. Med.* **360**, 985–993 (2009).
3. Haldar, P. *et al.* *N. Engl. J. Med.* **360**, 973–984 (2009).
4. Moore, W.C. *et al.* *Am. J. Respir. Crit. Care Med.* **181**, 315–323 (2010).
5. Willis-Owen, S.A., Cookson, W.O. & Moffatt, M.F. *Curr. Allergy Asthma Rep.* **9**, 3–9 (2009).
6. Moffatt, M.F. *et al.* *Nature* **448**, 470–473 (2007).
7. Hammad, H. & Lambrecht, B.N. *Nat. Rev. Immunol.* **8**, 193–204 (2008).
8. Hammad, H. *et al.* *Nat. Med.* **15**, 410–416 (2009).
9. Woodruff, P.G. *et al.* *Proc. Natl. Acad. Sci. USA* **104**, 15858–15863 (2007).
10. Woodruff, P.G. *et al.* *Am. J. Respir. Crit. Care Med.* **180**, 388–395 (2009).
11. Siddiqui, S. & Brightling, C. *Am. J. Respir. Crit. Care Med.* **180**, 383–384 (2009).

■ BENCH TO BEDSIDE

The early beginnings

Catherine Hawrylowicz & Kimuli Ryanna

The prevalence of allergic diseases has risen in recent decades¹, with up to 300 million people worldwide now estimated to suffer from asthma. Although susceptibility for these conditions has a hereditary element, given this rapid increase, changes in our environment are clearly also important. Infancy has historically been highlighted as the period when the risk is greatest for developing allergies. Strachan, following observations relating to family size and birth order, first suggested that infection in childhood has a protective effect through infections acquired from contact with older siblings².

Recently, studies in mice have implicated environmental factors even earlier in life, both *in utero*^{3,4} and possibly even before concep-

tion^{4,5}. These studies dovetail with epidemiological studies pointing to pregnancy as an important period of environmental influence^{1,6}, and they offer the potential to dissect the mechanisms associated with protection. Although in recent years a preventative role for regulatory T cells (T_{reg} cells) has gained support⁷, the mechanisms proposed in these studies often extend beyond T_{reg} cells to include altered maturation of innate immunity and induction of epigenetic changes.

The Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle study team has highlighted the protective influence of a farming environment not only in infancy, but additionally during pregnancy^{1,8}. The farmyard environment is associated with an increased exposure to microbes that signal to the innate immune response through pattern recognition receptors such as the Toll-like receptors (TLRs).

Endotoxin exposure during pregnancy in mice is known to protect the offspring against

allergen-induced airway disease, and a recent study by Conrad *et al.*³ extends these observations by using a cowshed-derived bacterium called *Acinetobacter lwoffii* F78. Maternal nasal exposure to the bacteria during pregnancy resulted in protection of the offspring from ovalbumin-induced airway inflammation and required intact TLR signaling in the mother. Exposure induced a transient local T helper type 1 (T_H1)-like proinflammatory response in maternal lung characterized by elevated tumor necrosis factor, interleukin-6 (IL-6) and IL-12 concentrations in bronchoalveolar lavage, followed by a systemic response associated with elevated serum IL-6 abundance. Moreover, the authors observed increased expression of TLR in maternal lung tissue, but not in the placenta³. Encouragingly, these data mirror studies showing increased innate receptor expression (for example, of TLR2, TLR4 and CD14) in human cord blood associated with farmyard exposure⁸. The Conrad study³ found no direct evidence for a role of T_{reg} cells, but rather for immune deviation toward a T_H1 -

Catherine Hawrylowicz and Kimuli Ryanna are at King's College London, UK Medical Research Council and Asthma UK Centre in Allergic Mechanisms of Asthma, Guy's Hospital, London, UK. e-mail: catherine.hawrylowicz@kcl.ac.uk or kimuli.ryanna@kcl.ac.uk

like proinflammatory response (Fig. 1). These findings support other studies⁹ that propose that delayed maturation of T_H1 responses in the neonate contribute to the development of atopy.

Another experimental model of allergic airway disease has raised the possibility of a role for epigenetic modification leading to effects in subsequent generations⁴. Hollingsworth *et al.*⁴ were intrigued by evidence that DNA hypomethylation in naive T cells enhances expansion of T_H1 cells and T_{reg} cells expressing the characteristic marker Foxp3. They hypothesized that affecting methylation status through a diet rich in methyl donors *in utero* may increase allergic airway disease⁴. They found that exposure of mice to such a diet during gestation, but not during lactation, increased the incidence of airway disease in the offspring⁴. Although less robust, the effects were also observed through paternal transmission to the F₂ generation.

The findings are in accordance with a separate study showing that protection from allergic disease can be conferred through the mother before conception⁵. Oral ovalbumin administration to female mice days before mating protected the progeny from subsequent induction of airway disease.

Hollingsworth *et al.*⁴ identified a number of differentially methylated genes after *in utero* supplementation with methyl donors, including excessive gene methylation of *Runx3* and reduced protein amounts of *Runx3*; these alterations were associated with increased airway disease severity. An independent study has highlighted a role for runt-related transcription factor-3 (*Runx3*), in knockout mice, in the prevention of airway disease¹⁰. *Runx3* has also recently been implicated in the induction and function of Foxp3⁺ T_{reg} cells¹¹. Methyl donors are present in toxic substances such as smoke, but an editorial accompanying the methyl donor paper advises caution in interpretation of the results, as other forms of methyl donors, such as folic acid, are advocated in pregnancy.

Together these emerging data suggest that prevention of allergy may require intervention during or even before pregnancy. Intervention during this period has the potential for effects not only on the first, but also on subsequent, generations⁴. A range of potential options for intervention is under consideration.

Epidemiological studies suggest that maternal diet in pregnancy is a prominent factor in prevention of atopy in early life. For example, low maternal intake of vitamin E, zinc and vitamin D during pregnancy have all been associated with an increase in recurrent wheeze and asthma in children up to 5 years of age⁶. Interventions under scrutiny include

Figure 1 Allergy *in utero*.

Epidemiological studies have identified a number of environmental factors during pregnancy, many of which are present in maternal diet, that can positively or negatively determine the likelihood of allergic disease in the offspring^{1–6,9}. Allergic disease is associated with development of inappropriate allergen-specific IgE and T_H2 responses, characterized by the synthesis of the disease-promoting cytokines IL-4, IL-5 and IL-13. Animal models of allergic airway disease have been used to investigate potential mechanisms by which these factors modify immune function in the progeny. These have been used to evoke a role for a range

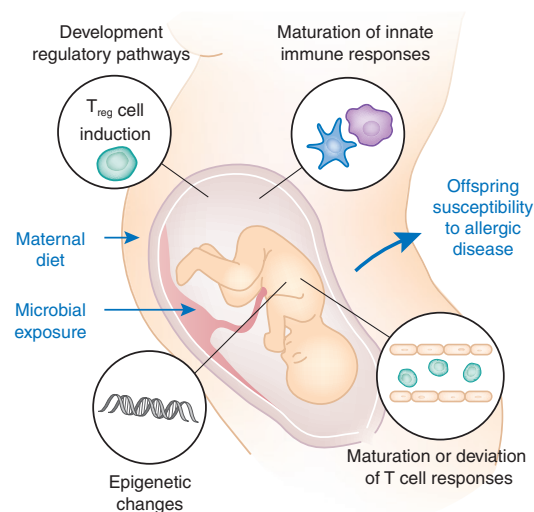
of mechanisms, including epigenetic changes, hereditary changes that do not involve alterations in DNA sequence, influencing regulatory and effector T cell responses⁴; triggering and appropriate maturation of innate immunity^{3,8}; deviation of the immune response from the T_H2-biased state of pregnancy toward a more balanced immune phenotype with a prominent T_H1 response (characterized by interferon- γ synthesis)⁹; and appropriate development of T_{reg} cell populations⁷.

the use of probiotics—dietary supplementation with live microorganisms. The aim is to modify the microbial environment to which the fetus is exposed and beneficially influence immune development, although, as yet, this approach has met with limited success in clinical studies¹².

Another approach involves modification of the immune response by various nutrients in the diet, such as certain lipids, vitamins and essential minerals. There is evidence that these nutrients modify T cell function⁶. One example is the extensive literature on the capacity of vitamin D to promote T_{reg} cells activity in mice and humans⁷. Vitamin D may have additional benefits in pregnancy, as replete vitamin D stores were recently shown to predict reproductive success in infertile women¹³. A number of registered trials are reviewing the role and timing of probiotics in the antenatal period, and trials are now in progress to assess the effect of nutritional supplementation such as vitamin D in mothers on the development of asthma and allergy in their offspring^{6,12}. Immunological analyses alongside these trials will be crucial to define relevant immune outcomes.

In addition to thinking about how to intervene, it is also important to think about when to do so. Epidemiological and experimental data suggest that researchers need to assess strategies in the period just before conception, throughout pregnancy, during lactation and in infancy.

Many questions remain. Can interventional strategies in the early prenatal period counteract any negative epigenetic changes that may



Katie Vicari

predispose to asthma? Will intervention in the later stages of pregnancy favor changes in the offspring's innate immune system, which may be of greater importance in the development of tolerance? Given the diverse effects of influences such as maternal diet, exposure to infection and environmental factors on both innate and adaptive immunity, will combinatorial strategies, such as the modification of gut flora with other dietary modifications, be required?

Identifying immunological mechanisms that explain these protective early life influences could hasten the possibility of disease prevention.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

1. Eder, W., Ege, M.J. & von Mutius, E. *N. Engl. J. Med.* **355**, 2226–2235 (2006).
2. Strachan, D.P. *Br. Med. J.* **299**, 1259–1260 (1989).
3. Conrad, M.L. *et al. J. Exp. Med.* **206**, 2869–2877 (2009).
4. Hollingsworth, J.W. *et al. J. Clin. Invest.* **118**, 3462–3469 (2008).
5. Polte, T., Hennig, C. & Hansen, G. *J. Allergy Clin. Immunol.* **122**, 1022–1030.e5 (2008).
6. Devereux, G. *Pediatr. Pulmonol.* **42**, 663–673 (2007).
7. Lloyd, C.M. & Hawrylowicz, C.M. *Immunity* **31**, 438–449 (2009).
8. Ege, M.J. *et al. J. Allergy Clin. Immunol.* **117**, 817–823 (2006).
9. Holt, P.G. & Sly, P.D. *Curr. Opin. Allergy Clin. Immunol.* **7**, 547–555 (2007).
10. Fainaru, O., Shseyov, D., Hantisteanu, S. & Groner, Y. *Proc. Natl. Acad. Sci. USA* **102**, 10598–10603 (2005).
11. Klunker, S. *et al. J. Exp. Med.* **206**, 2701–2715 (2009).
12. Johannsen, H. & Prescott, S.L. *Clin. Exp. Allergy* **39**, 1801–1814 (2009).
13. Ozkan, S. *et al. Fertil. Steril.* published online, doi:10.1016/j.fertnstert.2009.05.019 (2009).