K.L. Gatford, A.L. Wooldridge, K.L. Kind, R. Bischof, V.L. Clifton
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Pre-birth origins of allergy and asthma

KL Gatford[1,2], AL Wooldridge[1,2,4], KL Kind[1,3], R Bischof[5,6], VL Clifton[1,2,7]

[1]Robinson Research Institute; [2]Adelaide Medical School; [3]School of Animal and Veterinary Sciences, University of Adelaide; [4]School of Anatomy, Physiology and Human Biology, University of Western Australia; [5]Department of Physiology, Monash University; [6]Hudson Institute of Medical Research, Melbourne; and [7]Mater Medical Research Institute, University of Queensland

Corresponding author:

Dr KL Gatford

+61 8 8313 4518

kathy.gatford@adelaide.edu.au

Abstract: Allergy is a chronic disease that can develop as early as infancy, suggesting that early life factors are important in its aetiology. Variable associations between size at birth, a crude marker of the fetal environment, and allergy have been reported in humans and require comprehensive review. Associations between birth weight and allergy are however confounded in humans, and we and others have therefore begun exploring the effects of early life events on allergy in experimental models. In particular, we are using ovine models to investigate whether and how a restricted environment before birth protects against allergy, whether methyl donor availability contributes to allergic protection in IUGR, and why maternal asthma during pregnancy is associated with increased risks of allergic disease in children. We found that experimental intrauterine growth restriction (IUGR) in sheep reduced cutaneous responses to antigens in progeny, despite normal or elevated IgE responses. Furthermore, maternal methyl donor supplementation in late pregnancy partially reversed effects of experimental IUGR, consistent with the proposal that epigenetic pathways underlie some but not all effects of IUGR on allergic susceptibility. Ovine experimental allergic asthma with exacerbations reduces relative fetal size in late gestation, with some changes in immune populations in fetal thymus suggestive of increased activation. Maternal allergic asthma in mice also predisposes progeny to allergy development. In conclusion, these findings in experimental models provide direct evidence that a perturbed environment before birth alters immune system development and postnatal function, and provide opportunities to investigate underlying mechanisms and develop and evaluate interventions.

Key words: Pregnancy; Developmental programming; Experimental models; IUGR; Folic acid

1. Introduction

Several of the authors within this special issue have discussed the evidence that inflammation during pregnancy induces pregnancy complications, and the underlying mechanisms act via activation of toll-like receptor pathways. For example, maternal inflammatory signals induced by infectious and non-infectious stimuli are critical for normal labour and delivery and are implicated as causes of preterm labour. Intriguingly, the converse is also true, that exposures during gestation can predispose the progeny to later development of the inflammatory state of allergy. Rates of allergy are increasing rapidly, particularly in young children; the rate of hospitalisations for food-related anaphylaxis increased more than 5-fold in the 10 years from 1994-5 to 2004-5 in Australian children up to 4 years of age (Poulos *et al.* 2007). Understanding the aetiology of allergy and identifying preventative strategies is therefore increasingly important. The objectives of this review are to discuss key evidence for pre-birth origins of allergy and asthma from human cohorts and experimental models, in particular focussing on programming of allergy by three gestational exposures; intra-uterine growth restriction (IUGR), *in utero* methyl donor supply, and maternal allergy and inflammation. We conclude with suggestions for future research directions.

Commented [KG1]: please reference Chamley (PAMPs/maternal infection and preterm birth) & Robertson (DAMPs/TLRs in normal and preterm birth) papers in this issue

2. *In utero* exposures and later health

Associations between exposure to an adverse environment during pregnancy and infancy and later poor health were initially described at the regional level in seminal studies led by David Barker. Their subsequent work first linked individual birth and death records, and then progressed to studies of cardiometabolic outcomes in adults, and consistently demonstrated that individuals with low birth weights were at greater risk of poor cardiometabolic outcomes, including ischaemic heart disease and impaired glucose control (reviewed by Barker 1998). Subsequent studies of populations exposed to defined periods of famine revealed critical developmental periods *in utero* when different systems and their associated risks of later diseases were most susceptible to effects of maternal nutrient restriction, and showed that *in utero* exposures could change postnatal outcomes even in the absence of reduced birth weight (Roseboom *et al.* 2001). Adding to this evidence from opportunistic cohorts, studies in the Pima Indian population who have extremely high rates of diabetes in adulthood also provides strong evidence that the associations between gestational exposures and progeny health outcomes are not explained by genetics alone. In this population, siblings of mothers with diabetes are at >3-fold higher risk of diabetes themselves compared to siblings born before their mother was diagnosed with diabetes (Dabalea *et al.* 2000). Thus, exposures during critical windows of development have a lasting impact and impact adult health, a concept now referred to as 'developmental programming'. Since events early in life generally have the greatest impact on developmental trajectories, interventions early in life also have the greatest potential to improve adult health (Hanson and Gluckman 2014). To date, developmental programming of allergy has been far less studied than that of outcomes such as metabolic diseases.

We have recently reviewed evidence, largely in humans, for effects of perinatal exposures on the risks of allergy in progeny (Grieger *et al.* 2016). Parental and peri-conceptual factors such as low socio economic status, having a younger mother, and having older siblings, are each associated with reduced risk of developing allergy (Grieger *et al.* 2016). Having an older or obese mother, excessive maternal weight gain during pregnancy, being the first-born child and maternal smoking are associated with greater risk of developing allergy (Grieger *et al.* 2016). Restricted growth before birth appears to be protective against allergy, but is a risk factor for asthma. Most evidence suggests that maternal folic acid abundance in late pregnancy is positively associated with the risk of allergy in the offspring. Similarly, maternal inflammation due to allergy or asthma during pregnancy is a

susceptibility factor for later development of allergy in progeny. The evidence from epidemiological and experimental studies for programming of allergy by these three exposures is discussed below.

3. Protective effects of IUGR against allergy but not asthma

3.1 Evidence for IUGR as a protective factor from human cohorts

Overall, the evidence from human studies suggests that restricted growth *in utero* reduces the risk of allergy in infancy, although findings are variable. Data on allergic outcomes at later ages is limited and even more variable than that available for infants. In the ISAAC Phase III study, the risks of having had eczema by 6-7 years old were decreased overall in children with birth weights of <2.5 kg (OR 0.88, 95% CI: 0.82-0.96) and 2.5 to <3.0 kg (OR 0.94, 95% CI: 0.90-0.99) compared to the reference category with birth weights of 3.0 to <4.0 kg (Mitchell *et al.* 2014). When stratified for country of origin, the protective effect of low birth weight (LBW) for eczema was only significant for children from affluent countries, and not in those from non-affluent countries, implying interactions between fetal growth and other environmental exposures, and risks of hay fever were not related to birthweight (Mitchell *et al.* 2014). Strengths of this study include the large numbers of subjects (>162,000 children) and inclusion of centres from both developed and developing countries, but this data may be limited by use of absolute birth weights (not adjusted for gestational age), and parent recall/non-clinical diagnosis of allergy. In the PARIS cohort of 1860 French infants at 18 months old, high relative birth weight (3rd or 4th quartile of population) was associated with increased risks of sensitisation to food allergens, most commonly cow's milk and egg white, measured as elevated circulating allergen-specific IgE (Gabet *et al.* 2016). Risks of sensitisation to common aeroallergens were unaffected by birth weight in this cohort, however (Gabet *et al.* 2016).

Twin cohort studies can reduce confounding and variation due to genetics and environmental factors, and also support a protective effect of LBW on later allergy. Within the Swedish Twin Registry (Lundholm *et al.* 2010), rates of eczema increased with birth weight (for 500g increase in birth weight, OR 1.62, 95% CI: 1.27-2.06) although hay fever was not associated with birth weight. This relationship was strengthened (for 500g increase in birth weight, OR 3.83, 95% CI: 1.55-9.98) in co-twin analyses of twin pairs discordant for eczema, an approach that controls for gestational age and shared genetic and environmental factors (Lundholm *et al.* 2010).

Relationships between size at birth and asthma are generally in the opposite direction to those between size at birth and the allergic diseases discussed above. Using an absolute birth weight criterion of 2.5 kg to define LBW, the incidence of wheezing disorders (predominantly asthma), in childhood and adolescence was 60% higher in LBW than non-LBW in a recent meta-analysis of >1.7 million participants in 37 studies (Mebrahtu *et al.* 2015). Consistent with this, a recent meta-analysis of data from nearly 25,000 individuals in 24 European birth cohorts identified a 32% greater risk of asthma in LBW (< 2.5 kg) individuals compared to all others (den Dekker *et al.* 2016). Another meta-analysis, again of cohorts in developed countries, found similarly increased OR of asthma in children (↑28%) and adults (↑25%) for LBW (<2.5 kg) compared to all others (Mu *et al.* 2014). In the ISAAC Phase III study, asthma incidence was increased in children whose birth weights were <2.5 kg or 2.5 to <3 kg compared to the reference category of 3.0 to <4.0 kg, with a trend to stronger effects of LBW in affluent countries (Mitchell *et al.* 2014). In twin studies and co-twin analyses, lower birth weight is also associated with increased asthma risk (Örtqvist *et al.* 2009). The association between LBW and increased asthma risk probably reflect effects of a restricted *in utero* environment on lung development rather than allergy, since these studies do not differentiate allergic and non-allergic asthma, and the association with asthma is at least partly explained by poorer lung function (den Dekker *et al.* 2016). Although effects of LBW on asthma are likely confounded by gestational age,

and preterm birth is also a risk factor for asthma, the increased risk of asthma is also apparent in children born small for gestational age (SGA, birth weight <10th percentile, OR 1.18) as well as LBW (den Dekker *et al.* 2016). Unlike allergies, these meta-analyses suggest that high birth weight does not affect risk of asthma (Mebrahtu *et al.* 2015).

In addition to the lack of differentiation of allergic and non-allergic asthma, the mixed reports of associations between markers of growth *in utero* and later allergic outcomes in progeny probably also reflect the use of variable exposure markers; such as absolute birth weight, birth weight categories, LBW and SGA; and variability in the outcomes assessed and the age/s at which this has been done. Given this variation between studies and the lack of consensus in this area, we are conducting a systematic review of the evidence for relationships between birth weight or fetal growth rate and postnatal allergy (as per published protocol, Wooldridge *et al.* 2016). Although the available epidemiological data suggests that allergy is programmed by *in utero* exposures in humans, it does not enable clear separation of the effects of environmental factors and genetic susceptibility. The epidemiological evidence is also likely to be confounded by environmental factors such as nutrition that persist from prenatal to postnatal life, or by co-morbidities such as IUGR and preterm birth. Experimental models have therefore been used to directly test effects of induced IUGR on progeny allergy, and may in the future allow evaluation of intervention strategies to reduce allergy risk.

3.2 Chronic experimental IUGR reduces allergic sensitisation

Allergic sensitisation has been reported in only a few experimental models of IUGR to date, with variable effects possibly reflecting the cause of IUGR (and hence different fetal exposures) as well as different developmental timings of restriction. In Wistar rats, maternal nutrient restriction to 50% of *ad libitum* intake from mating until delivery induces a severe IUGR phenotype, reducing birth weight of pups by 32-34%. Allergic responses of young adult progeny to airway allergen challenge, including OVA-specific IgE production, inflammatory cell airway infiltration, mucus secretion and collagen deposition were attenuated in progeny of feed-restricted mothers compared to control progeny (Landgraf *et al.* 2008, Landgraf *et al.* 2012). Lung cytokine and transcription factor gene expression patterns in allergen-challenged progeny were also altered, suggesting a shift from Th1 to Th2 immune responses following *in utero* exposure to maternal undernutrition (Landgraf *et al.* 2012). In contrast, allergic responses to OVA sensitisation and a 2-week OVA inhalation exposure were increased rather than decreased in IUGR rat progeny (birth weight <10th centile of control progeny) when induced by a similar maternal undernutrition protocol throughout pregnancy in Sprague-Dawley rats (Xu *et al.* 2014). This accentuated allergic response after OVA challenge occurred in conjunction with increased lung endothelin-1 (ET-1) protein and gene expression, together with increased histone acetylation but unchanged methylation of the *ET-1* promoter, in IUGR compared to control progeny (Xu *et al.* 2014). Causality of the epigenetic changes and increase ET-1 expression in enhanced allergic responses of these IUGR progeny has not yet been demonstrated. Why effects of maternal undernutrition on allergic susceptibility of progeny differ between these two sets of studies is not clear, but might relate to rat strain, progeny sex or differences in sensitisation dose or continuous vs intermittent OVA challenge protocols. A milder reduction of 17% in neonatal weight induced using a maternal pregnancy stress protocol in mice (24 h sound stress at d 12 and d 14 of pregnancy) was associated with increased allergic responses in adult progeny (Pincus-Knackstedt *et al.* 2006). Conversely, maternal noise-induced stress protocols (hourly exposure each day from d 15 to 21 of pregnancy) that did not alter pup size at birth reduced delayed hypersensitivity reaction to bovine serum albumin in sensitised male and female progeny (Sobrian *et al.* 1997). Further studies

appear needed to clarify the effects of IUGR on allergic susceptibility in rodents and to determine which aspects of the *in utero* environment alter immune development and predispose to allergy.

In humans, IUGR is often associated with impaired placental function, and this can be mimicked experimentally by pre-mating removal of the majority of placental attachment sites before mating in sheep (placental restriction, PR), which reduces placental size and function (Alexander 1964, Robinson *et al.* 1979). We have applied established protocols for systematic sensitisation to allergens and cutaneous allergen challenges in this species to evaluate effects of PR on susceptibility to allergy (Bischof *et al.* 2008). In our recent studies, PR reduced birth weight by 20%, and decreased delayed cutaneous hypersensitivity reactions to OVA despite increased IgE responses to allergens after sensitisation to OVA and house dust mite (Wooldridge *et al.* 2014). Acute cutaneous inflammatory responses to histamine correlated positively with birth weight in singleton progeny of this cohort (Wooldridge *et al.* 2014). We have since found that mast cell density in skin is not reduced in the adult PR progeny (Wooldridge et al., unpublished). We therefore hypothesise that loss of mast cell function explains the suppressed cutaneous delayed hyper-sensitivity inflammatory responses in the presence of normal or exaggerated IgE responses to allergens in PR sheep, but this requires direct testing. Overall, the balance of evidence from experimental models suggests that chronic IUGR induced by reduced nutrient supply to the fetus is protective against allergy, consistent with the associations between low birth weight and reduced incidence of allergy reported in children.

4. *In utero* methyl donor metabolism in developmental programming of allergy

4.1 Evidence for methyl donor abundance as an asthma and allergy risk factor from human cohorts

Adequate maternal folate (Vitamin B_9) status before conception and in the first few weeks of pregnancy is critical for proper development of the embryonic neural tube. Periconceptional folic acid supplementation is an extremely effective preventative measure, reducing the risk of neural tube defects (NTDs) by at least 40% (Blom 2009), and health authorities in most countries and the World Health Organisation therefore recommend intakes of folic acid supplementation of 0.4-0.5 mg/d from at least a month before conception and during the first trimester (Gomes *et al.* 2016). Many pregnancies are unplanned, however, and these women are unlikely to know they are pregnant until after development of the neural tube during the 3rd and 4th weeks after conception. Voluntary and mandatory food fortification has therefore been implemented in many countries over the past 15 years to increase folate status in all women of reproductive age, and has further reduced rates of NTDs (Bower *et al.* 2009). Women at high risk of delivering a baby with an NTD, including those whose previous children have had NTDs, are recommended to consume 10-fold higher doses of 4-5 mg/d folic acid periconceptionally (Gomes *et al.* 2016). Randomised clinical trials are also evaluating efficacy of high folic acid doses (comparing 0 and 4 mg/d from before pregnancy to 12 weeks post-conception, followed by 0.2 or 0.8 mg/d for the remainder of pregnancy) in prevention of all congenital malformations, not just NTDs (Bortolus *et al.* 2014).

The evidence collated in several recent systematic reviews is that maternal folic acid supplementation at the usual doses of 0.4-0.5 mg/d during the periconceptional period before conception and during the first trimester of pregnancy is not associated with increased rates of childhood asthma (Blatter *et al.* 2013, Crider *et al.* 2013, Brown *et al.* 2014). There is some evidence that higher doses of folic acid during pregnancy are associated with asthma, based on linkage of maternal and children pharmacy dispensing data for >39 000 pregnancies in the Netherlands (Zetstra-van der Woude *et al.* 2014). Similar associations are evident in those dispensed high-dose

folic acid in either the first or third trimester alone (Zetstra-van der Woude *et al.* 2014). There is also some evidence to support the original suggestion from study of a prospective birth cohort, that maternal consumption of folic acid supplements specifically in late pregnancy may increase risks of childhood asthma (Whitrow *et al.* 2009). Maternal consumption of folic acid supplements in late gestation is associated with 6-26% greater risk of childhood asthma/wheeze in progeny (Brown *et al.* 2014). Effects of folic acid supplementation on incidence of allergic sensitisation and eczema in childhood vary between studies, with some finding increased risk and others no effect (Brown *et al.* 2014), and more data is needed to characterise effects of supplement at specific periods of pregnancy and at different doses. Tuokkola and colleagues recently reported that in a cohort of 2327 children in the Finnish Type 1 Diabetes Prediction and Prevention study, maternal folic acid supplement use but not dietary folate intake in the 8th month of pregnancy was associated with 40% greater risk of cow's milk allergy in 5 year-old children (Tuokkola *et al.* 2016). This suggests that maternal folic acid supplementation in late gestation is likely to predispose to progeny to later allergic disease in general, and not specifically asthma. Any changes to dietary recommendations about folic acid supplementation in pregnancy need to be made with care, in order not to confuse women about the benefits of peri-conceptional supplementation in reducing NTDs. Additional information is therefore required, including childhood allergic outcomes in trials of high-dose maternal folic acid, to clearly define the impact of high and late pregnancy consumption of folic acid on allergic outcomes, potentially providing the opportunity to intervene at a population level to decrease allergic disease incidence.

4.2 Experimental manipulation of 1-carbon pathways and progeny allergy

The strongest experimental evidence for a role of methyl donor exposure *in utero* in allergic susceptibility comes from a study where female mice were fed diets containing high (HMD) or low (LMD) levels of methyl donors and co-factors important in 1-carbon metabolism (folic acid, vitamin B_{12}, choline, l-methionine, zinc, and betaine) from 2 weeks before mating until weaning of the progeny (Hollingsworth *et al.* 2008). Compared to the LMD group, feeding HMD throughout pregnancy increased the severity of allergic airway disease (Th2-type immune responses) not only in the progeny exposed to this diet *in utero* (F1 generation), but also in the F2 generation (Hollingsworth *et al.* 2008). DNA methylation at multiple gene loci differed between HMD and LMD progeny, including greater methylation of *Runx3* with decreased *Runx3* gene and protein expression in HMD progeny, potentially causal in greater allergic susceptibility since this gene negatively regulates allergic airway disease (Hollingsworth *et al.* 2008). A number of methylated genes are also important determinants of T cell lineage, providing another pathway for effects of methyl donor metabolism on immune phenotype. For example, demethylation of *FoxP3* correlates with greater expression of *FoxP3* in whole cord blood, as well as with circulating T_{reg} cell numbers and suppressive activity of T_{regs} in culture of mononuclear cells isolated from cord blood and challenged with common allergens (Liu *et al.* 2010).

Our findings that PR protects progeny against allergic sensitisation (Wooldridge *et al.* 2014), discussed above, are also consistent with the hypothesis that decreased methyl donor abundance *in utero* may alter methylation of key genes to initiate a trajectory of immune system development that is subsequently less susceptible to developing allergy. In rodent models of PR, fetal 1-carbon donor abundance is decreased, 1-carbon pathway enzyme expression is altered and this is associated with hypomethylation of DNA and increased histone acetylation in multiple tissues (MacLennan *et al.* 2004, Ke *et al.* 2006, Park *et al.* 2008). Consistent with the hypothesis that reduced placental methyl donor transport to the fetus protects against allergy in the PR sheep, when we supplemented PR ewes with methyl donors and cofactors in the last month of their five month

gestation, the protective effects of PR against cutaneous delayed-type hypersensitivity after allergen sensitisation were partially lost (Wooldridge et al., unpublished). Effects of PR on antibody responses to allergen sensitisation were not altered by maternal methyl donor supplementation, however (Wooldridge et al., unpublished). We are currently investigating effects of our PR and maternal methyl donor supplementation on 1-carbon metabolism in our ovine models to further evaluate the potential role of methyl donors in programming of allergy.

5. Maternal asthma and allergy during pregnancy increase allergic susceptibility in progeny

5.1 Evidence for maternal asthma and allergy during pregnancy as allergy risk factors from human cohorts

Maternal asthma is a common gestational exposure, affecting ~12% of singleton pregnancies in an Australian cohort (Clifton *et al.* 2009). Maternal asthma worsens during pregnancy in ~50% of women, and 20% of asthmatic women undergo exacerbations requiring medical intervention (Murphy *et al.* 2005, Murphy *et al.* 2006). Asthma during pregnancy substantially increases risks of adverse pregnancy outcomes, including preeclampsia (↑54%), preterm birth (↑41%), SGA (↑22%), and LBW (↑46%) (Murphy *et al.* 2011). Risks of adverse neonatal outcomes including admission to neonatal intensive care (↑12%), respiratory distress syndrome (↑9%) and transient tachypnoea of the newborn (↑10%) are also increased when the mother has asthma, even after correction for prematurity as a comorbidity (Mendola *et al.* 2014).

In addition to these short-term adverse outcomes, there is good epidemiological evidence suggesting that exposure to maternal asthma or allergy before birth increase risks of the same conditions in children. Maternal asthma is consistently a stronger risk factor for childhood asthma than paternal asthma, implying that the maternal contribution is not only genetic, but that the *in utero* and possibly lactational environment also contribute to risk (Lim *et al.* 2010). Exposure to active maternal allergy is associated with increased risks of multiple childhood allergies, although interestingly, not with childhood asthma. In the PAULA study cohort of 526 children born in greater Munich in Germany, atopic symptoms in the mother during pregnancy were associated with >175% greater odds of food sensitisation in children within the first year of life, 60% greater odds of eczema (atopic dermatitis) in the first two years of life, and ~100% greater odds of hay fever (allergic rhinitis) at 4-5 years of age (Illi *et al.* 2014). Increased odds ratios for eczema in the first two years of life and of hay fever at 4-5 years of age were also observed in a sub-analysis of children from atopic mothers, also supporting the hypothesis that these relationships reflect programming by environmental factors in addition to genetic susceptibility (Illi *et al.* 2014). Although maternal atopic symptoms during pregnancy were not associated with altered odds of asthma before 4-5 years of age, nor with current wheeze at 4-5 years of age in the children, increased frequency of maternal infection with common colds during pregnancy was associated with more than double the odds for childhood asthma (Illi *et al.* 2014). Together, this evidence implicates *in utero* exposure to maternal inflammation - induced by maternal allergy, asthma or infection - as a factor that increases susceptibility of progeny to allergic disease postnatally. Altered T cell development is implicated in programming of allergic susceptibility by exposure to maternal allergy *in utero*. Compared to neonates born from non-allergic women, neonates from allergic women have a higher proportion of Th2 cells and lower ratio of T_{reg} to Th2 cells in cord blood (Fu *et al.* 2013). In the same study, low Th1:Th2 and T_{reg}:Th2 cell ratios in cord blood predicted increased risk of eczema development in the infants by two years of age (Fu *et al.* 2013). DNA in peripheral blood is also differentially methylated in peripheral blood of 1 year-old infants born to mothers with asthma, compared to infants of non-

asthmatic mothers, and some of the changes in DNA methylation correlate with characteristics of asthma and allergy severity in the mother or with infant circulating immune cell abundance (Gunawardhana *et al.* 2014). Whether these methylation changes at birth predict subsequent allergic outcomes in children is yet to be determined. The effects of maternal asthma and exacerbations in pregnancy on pregnancy outcomes and fetal and placental responses differ depending on whether the fetus is male or female (Clifton *et al.* 2009). Intriguingly, within the Isle of Wight Birth cohort, associations between allergy in parents and children were parent-of-origin specific and differed according to the sex of the child, such that maternal allergy was associated with increased risk in girls, and paternal allergy was associated with increased risk in boys (Arshad *et al.* 2012). Whether effects of maternal asthma and allergy are sex-specific requires confirmation in other cohorts, and if confirmed, further study to determine the extent to which this reflects effects of imprinted genes or sex-specific effects of the *in utero* environment on fetal immune development and allergic susceptibility.

5.2 Experimental allergy and asthma in the mother pre-dispose progeny to allergy

To date, the hypothesis that susceptibly to allergic disease is programmed by *in utero* exposure to maternal atopic states has only been adequately tested in mice. We have recently developed an ovine model of maternal allergic asthma which will enable this question to be evaluated in a large animal model and allow direct studies of fetal responses and longitudinal studies of individual progeny. This model will also be described below.

In the mouse, maternal allergic asthma before and during pregnancy increases susceptibility of pups to allergy, predisposing them to allergic responses to sensitisation (Hamada *et al.* 2003). This is a systemic response, since pups are more likely to develop allergic responses to novel antigens as well as after sensitisation with ovalbumin, the allergen used to induce maternal allergy (Hamada *et al.* 2003). At least in this mouse model, exposure during gestation or lactation was sufficient to induce allergic susceptibility in progeny, suggesting circulating inflammatory cells or signals in the mother can be transmitted to progeny across the placenta or in breastmilk (Leme *et al.* 2006). Transfer of allergen-specific T-cells from donor mice to non-sensitised dams followed by airway allergen exposure during pregnancy also increased risk of allergic asthma in mouse progeny without causing overt maternal asthma, showing that the presence and stimulation of allergen-specific T cells during pregnancy are sufficient to program allergic susceptibility in progeny (Hubeau *et al.* 2006). Only induction of an allergic (Th2-biased) immune response to OVA increases progeny susceptibility to allergic sensitisation. If dams are sensitised to OVA using a protocol that induces a Th1-biased immune response, then pups are actually protected against allergic sensitisation to OVA, although protection by the maternal Th1 response is allergen-specific, in contrast to induction of susceptibility (Matson *et al.* 2007). Consistent with this protective effect of non-allergic maternal allergen exposures, maternal airway OVA exposure from early pregnancy until delivery, which did not induce maternal allergy, induced IL-10 and T_{reg}-mediated immune tolerance to OVA in progeny that inhibited their allergic responses to OVA-sensitisation into adulthood (Gerhold *et al.* 2012). In a single study in dogs, maternal and paternal sensitisation to ragweed before mating was associated with increased circulating antibody responses and asthmatic-type lung responses to inhaled ragweed in progeny. This study is limited, however, by use of pups from only two litters in each group and potential effects of maternal ragweed exposure during lactation (Barrett *et al.* 2003). Together with the human data, these results in experimental models are consistent with the hypothesis that exposure to maternal allergy *in utero*, but not allergen exposure in the absence of allergy, increases the allergic susceptibility of progeny, and that this is not specific to the *in utero*-exposed allergen/s.

In order to directly evaluate the acute fetal and long-term progeny effects of maternal allergic asthma, and to enable evaluation of the effects of clinical and experimental interventions on these, we have recently developed an ovine model of maternal allergic asthma in pregnancy (Clifton *et al.* 2015). Sheep are sensitised systematically by repeated immunisation with allergen, followed by repeated airway challenges with aerosolised allergen, utilising a protocol that induces an allergic asthmatic phenotype in non-pregnant sheep (Bischof *et al.* 2003, Bischof *et al.* 2008). We mated ewes that had been sensitised and commenced airway challenges to house dust mite prior to pregnancy, and continued airway challenges with house dust mite throughout pregnancy (Clifton *et al.* 2015). These pregnant ewes developed characteristics of allergic asthma including increased lung resistance, progressive increases in the eosinophil influx induced by airway allergen challenges, and increased deposition of smooth muscle around lung airways (Clifton *et al.* 2015). The 12% reduction in relative fetal weight in late pregnancy in this model is consistent with effects of maternal asthma in human pregnancy, although additional studies are needed to determine whether fetal responses to maternal allergic asthma in sheep are sex-dependent as occurs in humans (Clifton *et al.* 2009, Clifton *et al.* 2015). We have begun to study the effects of maternal allergic asthma on fetal immune phenotype in this model. To date, the main effect we have observed is that fetuses from allergic ewes had a greater proportion of CD44-positive lymphocytes in thymus than control fetuses, with a similar trend in the lymphocyte population isolated from spleen (Wooldridge et al., unpublished data). This cell adhesion molecule marker is involved in lymphocyte adhesion to endothelial cells via hyaluronic acid and this interaction is essential for migration of activated T cells into sites of inflammation (DeGrendele *et al.* 1996, DeGrendele *et al.* 1997). Blocking CD44 action in a mouse model of airway allergic inflammation prevented or attenuated many of the inflammatory responses to airway allergen challenge including eosinophil and lymphocyte accumulation in lung, antigen-induced increases in Th2 cytokines and chemokines in lung liquid and antigen-induced airway hyper-responsiveness (Katoh *et al.* 2003). Anti-CD44 antibody treatment also inhibits the cutaneous delayed-type hypersensitivity in a murine model of contact allergy (Camp *et al.* 1993), consistent with the importance of CD44 in allergic inflammation. If the elevated CD44 expression in lymphocytes we see in late gestation fetuses in our ovine model of maternal allergic asthma persists, it may therefore predispose the progeny to allergic inflammation postnatally. The availability of this large animal model of maternal allergic asthma, where allergic sensitisation and tissue and molecular responses can be investigated in the same animals over time, will allow us to investigate these potential mechanisms for developmental programming of allergy.

6. Conclusions

On balance, the available epidemiological and experimental evidence suggests that prenatal chronic restriction of fetal growth reduces later risks of allergy, while elevated methyl donor availability in late pregnancy or active maternal asthma and allergy during pregnancy increase allergy susceptibility of progeny. Approaches such as discontinuing maternal folic acid supplementation during late pregnancy and tight control of maternal asthma and allergy in pregnancy should be evaluated as potential approaches to reduce the incidence of allergic diseases in children. Additional experimental studies are needed to identify underlying mechanisms for programming of allergic susceptibility by these and other exposures before birth, particularly for conditions such as IUGR which have other adverse effects.

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