

Association of maternal anti-HLA class II antibodies with protection from allergy in offspring

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Abstract

Background: Recent studies have suggested that the birth order effect in allergy may be established during the prenatal period and that the protective effect may originate in the mother. HLA class II disparity between mother and foetus has been associated with significantly increased Th1 production. In this study, we investigated whether production of HLA antibodies 4 years after pregnancy with index child is associated with allergic outcomes in offspring at 8 years.

Methods: Anti-HLA class I and II antibodies were measured in maternal serum ($n = 284$) and levels correlated to numbers of pregnancies and birth order, and allergic outcomes in offspring at 8 years of age.

Results: Maternal anti-HLA class I and II antibodies were significantly higher when birth order, and the number of pregnancies were larger. Anti-HLA class II, but not class I antibodies were associated with significantly less atopy and seasonal rhinitis in the offspring at age 8 years. Mothers with nonatopic (but not atopic) offspring had a significant increase in anti-HLA class I and II antibodies with birth order.

Conclusion: This study suggests that the 'birth order' effect in children may be due to parity-related changes in the maternal immune response to foetal antigens. We have observed for the first time an association between maternal anti-HLA class II antibodies and protection from allergy in the offspring. Further work is required to determine immunologically how HLA disparity between mother and father can protect against allergy.

The prevalence of atopy and some allergic disease such as hayfever is consistently lower in children from large families or of higher birth order (1). These 'sibship effects' were historically explained through the hygiene hypothesis (2). More recent studies raise the alternative and intriguing possibility that the 'birth order' effect may be established during the prenatal period and that the protective effect of a higher number of siblings originates in utero (3). Mothers of higher parity have lower levels of total IgE as do their newborn children (3). Atopic mothers have fewer offspring compared with nonatopic mothers (4, 5) and a higher number of pregnancies may be associated with loss of atopy in the mother (6).

During pregnancy, the interaction between the mother and foetus can modify both maternal and foetal cytokine production and in particular IFN- γ levels (7, 8) with implications for the subsequent development of allergic disease in the

child (9). Lower levels of IFN- γ in early life are associated with the development of allergic manifestations in early childhood (10–12). The IFN- γ response to foetal cells is lower in allergic than nonallergic mothers (7, 8). In contrast, a higher number of pregnancies are associated with increased levels of maternal IFN- γ in response to foetal cells (9). Furthermore, mismatch of HLA between mother and foetus as measured by the presence of anticlass II (but not class I) antibodies (9) and HLA-DR β 1 mismatch determined by HLA typing (8) is also associated with stronger foetal IFN- γ alloantigen. Thus, the raised levels of IFN- γ associated with a higher number of pregnancies and HLA mismatch between mother and foetus may be associated with a lower risk of allergic disease in the developing child.

The aim of this analysis was to determine whether maternal production of HLA antibodies arising from HLA

mismatch between mother and foetus is associated with allergic outcomes in their offspring at age 8 years.

Material and methods

Population

Details of the Ashford birth cohort have been reported (13) and are summarized in Fig. 1. Between 1993 and 1995, all newly pregnant women who presented at one of three GP surgeries in Ashford, Kent were approached to participate in a longitudinal study. A total of 658 (93% of those eligible) agreed, and 642 babies were subsequently born to 625 women. During pregnancy, skin prick tests were performed on all but three mothers, with atopy defined as at least one mean weal diameter ≥ 3 mm larger than the response to a saline control; three common allergens were used (pollen mixture, *Dermaphagoides pteronyssinus* and cat fur; ALK, Allergopharma, UK). Families were revisited yearly until the child's sixth birthday and then again at the age of 8 years. Maternal blood samples were collected when the child was 4 years of age ($n = 523$; 84%). At age eight, allergic outcomes were measured in the children through a standardized questionnaire administered to their parents (14). In this way, we collected

information for 92% of the children on seasonal rhinitis defined as a positive response to the question 'In the past 12 months, has your child ever had a problem with sneezing or a runny or blocked nose when s/he did not have a cold or the flu?' along with a record of this occurring in any of the months from March to September inclusive (15). Asthma was defined as a positive response to the question 'Has your child had wheeze in the last 12 months?' A child was considered to have atopic eczema, according to the UK diagnostic criteria, if they experience an itchy skin in the past 12 months and had at least three of the following: a history of flexural involvement, a history of a generally dry skin, a history of allergic disease or visible dermatitis (16). Through skin prick tests (85% of children at age eight), we established the presence of atopy, using the same antigens as used earlier for their mothers but defining a positive response as one with a mean weal diameter of 2 mm or more. The definition of atopy is restricted to three common airborne allergens and excludes sensitization to other allergens such as moulds and food.

Our original request for informed consent did not include the measurement of HLA antibodies necessary for this study. After approval by the Royal Brompton Hospital/National Heart and Lung Institute ethics committee, we sought retrospective consent which was provided by 442 women (85% of those who gave a blood sample). Due to the difference in time between the birth of the children and the later collection of the mother's blood sample, all analyses relating to maternal antibodies have been restricted to those with no further children born in this interval ($n = 298$; 67%). For most of these mothers ($n = 269$; 90%), further information relating to all pregnancies, including unsuccessful pregnancies, was available.

Anti-HLA antibody measurement

Sera were tested for the presence of HLA-specific antibodies using the Labscreen Mixed assay (One Lambda, Canoga Park, CA, USA) as previously described (17). This method detects both complement fixing and noncomplement fixing HLA antibodies using Luminex microbeads coated with purified HLA class I and class II molecules. Sera were incubated with the beads for 30 min, followed after washing, by incubation with anti-human IgG phycoerythrin (PE) conjugated monoclonal antibody for 30 min. Median fluorescence intensity levels were measured and positivity determined using manufacturers' software with predetermined cut-off values calculated from laboratory validation procedures.

Anti-HLA results were available for 284 (95%) of eligible maternal samples.

Statistical methods

We compared the representativeness of those mothers included in our analysis ($n = 298$) with the original cohort ($n = 442$) (those with retrospective ethical consent) using a t-test for maternal age and chi-squared tests for the other factors (Table 1). Associations between the presence or absence of maternal anti-HLA antibodies and allergic outcomes at age 8 years were investigated using chi-squared tests. Associations

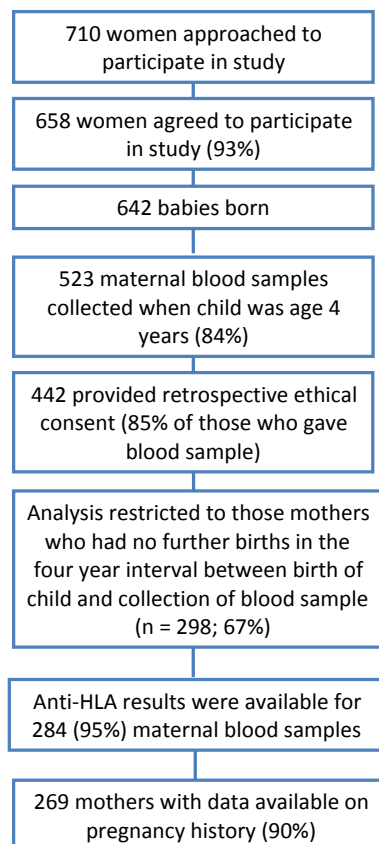


Figure 1 'Asthma in Ashford' study: cohort and population for this analysis.

between anti-HLA antibodies and birth order or number of pregnancies were assessed using chi-squared tests for trend.

Results

Characteristics of population

Within this study, we restricted anti-HLA analyses to mothers who had no further children born following the birth of the index child. We compared the representativeness of those mothers included in our analysis with those mothers in the original cohort (Table 1). Perhaps not surprisingly, mothers included in the analysis were more likely to have higher parity and already have had a child at recruitment which suggests that their families were more likely to be complete. There appeared to be little difference between the whole cohort and those included in our analysis in terms of maternal age, maternal atopy, child's atopy or child's seasonal rhinitis.

Prevalence of anti-HLA class I and II antibodies in maternal serum: effects of maternal atopy and pregnancy

A total of 129 (50%) and 65 (24%) mothers had detectable anti-HLA class I and II serum antibodies, respectively. There was a nonsignificant trend for atopic mothers to have a lower prevalence of anti-HLA class II serum antibodies (18.6%) compared with nonatopic mothers (26.4%) ($P = 0.14$).

There were clear, statistically significant linear trends between the proportions of mothers with detectable anti-HLA class I and II antibodies and both their child's birth order (P (trend) < 0.001 for both class I and II) and the number of their previous pregnancies (P (trend) = 0.02 for class I and 0.003 for class II) (Table 2). Similar linear trends were observed when data were stratified according to the atopic status of the mother (Table 2).

Effect of child's atopic status on relationship between maternal anti-HLA antibodies and number of pregnancies

The relationship between the prevalence of maternal anti-HLA antibodies and birth order was much stronger when the

child, at age 8 years, was nonatopic compared with atopic. Mothers with a nonatopic child had a significant linear increase in anti-HLA class I and II antibodies with birth order ($P < 0.001$ and $P = 0.002$, respectively; Fig. 2). In contrast, the prevalence of antibodies in mothers with an atopic child did not significantly change with birth order for either anti-HLA antibody class ($P = 0.35$ and $P = 0.40$, respectively; Fig. 2).

Effect of presence of anti-HLA class I and II antibodies in maternal serum with allergic outcomes in the offspring

The detection of maternal anti-HLA class II antibodies was associated with significantly less atopy ($P = 0.05$), less seasonal rhinitis ($P = 0.05$) and borderline statistical significance with asthma in the offspring at age eight (Table 3). Anti-HLA class I antibodies in maternal serum were associated with a reduction in seasonal rhinitis in the child although with borderline statistical significance ($P = 0.08$; Table 3). Maternal anti-HLA class I antibodies were not associated with either atopy or asthma in the child at age eight (Table 3). Neither maternal anti-HLA class I nor II antibodies were associated with eczema (data not shown).

We examined the association between maternal HLA class I and II antibodies and birth order upon the child's allergic status (adjusting for maternal and paternal atopy) using logistic regression. Both HLA class I and II antibodies tended towards being protective against atopy (OR = 0.79 (95% CI 0.40–1.58) and OR = 0.51 (95% CI 0.21–1.24), respectively), seasonal rhinitis (OR = 0.68 (95% CI 0.35–1.31) and OR = 0.62 (95% CI 0.27–1.43), respectively) and asthma (OR = 0.57 (95% CI 0.24–1.3) and OR = 0.44 (95% CI 0.14–1.34), respectively), although none reached statistical significance. Increasing birth order had little effect on atopy in models including HLA class I and II (OR = 0.91 (95% CI 0.63–1.33) and OR = 0.98 (95% CI 0.68–1.41), respectively) but this association tended towards being protective with seasonal rhinitis (OR = 0.67 (95% CI 0.45–0.99) and OR = 0.65 (95% CI 0.43–0.96), respectively).

We examined the intervening variables between birth order and either atopy or seasonal rhinitis using partial correlation.

Table 1 Representativeness of included participants

	Mothers (all; $n = 442$)	Mothers included in analysis (no further births; $n = 298$)	P -value
Parity at recruitment			
0	195 (44.1%)	95 (31.9%)	0.004 (trend: 0.003)
1	154 (34.8%)	127 (42.6%)	
2+	93 (21.0%)	76 (25.5%)	
Maternal age; mean (SD) years	28.2 (4.7)	28.7 (4.7)	0.13
Any children prior to recruitment; n (%)	247 (55.9%)	203 (68.1%)	0.001
Maternal atopy; n (%)	155 (35.1%)	103 (34.6%)	0.94
Child atopy; n (%)	81 (19.3%)	55 (19.6%)	0.92
Child seasonal rhinitis; n (%)	91 (20.7%)	59 (19.9%)	0.85

Table 2 Prevalence of detectable anti-HLA antibodies in maternal serum by birth order and number of pregnancies

	Mother atopic and nonatopic Anti-HLA antibodies in maternal serum		Mother nonatopic (<i>n</i> = 195) Anti-HLA antibodies in maternal serum		Mother atopic (<i>n</i> = 103) Anti-HLA antibodies in maternal serum	
	Class I +ve	Class II +ve	Class I +ve	Class II +ve	Class I +ve	Class II +ve
Birth order						
1st	30/81 (37.0%)	12/87 (13.8%)	21/47 (44.7%)	8/50 (16.0%)	9/34 (26.5%)	4/37 (10.8%)
2nd	54/111 (48.7%)	27/120 (22.5%)	31/67 (46.3%)	18/76 (23.7%)	23/44 (52.3%)	9/44 (20.5%)
3rd or later	45/66 (68.2%)	26/68 (38.2%)	33/48 (68.9%)	21/52 (40.4%)	12/18 (66.7%)	5/16 (31.3%)
<i>P</i> (trend)	<0.001	<0.001	0.02	0.01	0.003	0.07
Number of pregnancies						
1	18/45 (40.0%)	7/47 (14.9%)	15/29 (51.7%)	5/30 (16.7%)	3/16 (18.8%)	2/17 (11.8%)
2	41/87 (47.1%)	18/93 (19.4%)	24/51 (47.1%)	14/57 (24.6%)	17/36 (47.2%)	4/36 (11.1%)
3	27/53 (50.9%)	18/59 (30.5%)	18/37 (48.7%)	14/43 (32.6%)	9/16 (56.3%)	4/16 (25.0%)
4+	33/52 (63.5%)	19/51 (37.3%)	21/31 (67.7%)	13/32 (40.6%)	12/21 (57.1%)	6/19 (31.6%)
<i>P</i> (trend)	0.02	0.003	0.20	0.02	0.03	0.05

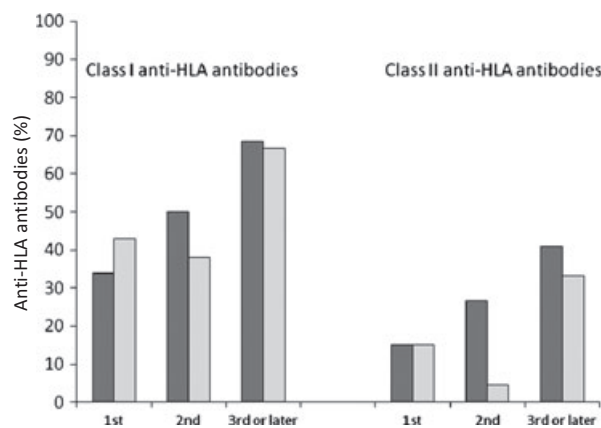


Figure 2 Prevalence of anti-HLA antibodies in maternal serum stratified by child's atopic status at age 8 years. Darker bars are for nonatopic children, lighter bars for atopic children. Mothers with a nonatopic child had a significant linear increase in anti-HLA class I and II antibodies with birth order ($P < 0.001$ and $P = 0.002$, respectively). Mothers with an atopic child did not have a significant linear increase with birth order for either anti-HLA class I or II antibodies ($P = 0.35$ and $P = 0.40$, respectively).

Birth order and maternal anti-HLA class I and II antibodies were found to be significant when either child's atopic status (partial correlation was 0.13 and 0.11, respectively, $P < 0.05$) or seasonal rhinitis (partial correlation was 0.11 and 0.10, respectively, $P < 0.05$) was taken into account. The partial correlation between atopy and maternal HLA class I and II antibodies did not reach statistical significance after adjusting for birth order.

Discussion

Within a birth cohort with a high rate of participation and retention, we confirmed the relationship between detectable maternal anti-HLA antibodies and both the birth order of their child and the number of their own pregnancies (18–20).

Table 3 Maternal anti-HLA antibodies by allergic outcomes in their child at age 8 years

Maternal anti-HLA antibodies	Child's allergic status at age 8 years		
	Atopic (weal >2 mm)	Seasonal rhinitis	Asthma (wheezy in past 12 months)
Class I			
–ve	28/123 (22.8%)	34/129 (26.4%)	18/129 (14.0%)
+ve	23/120 (19.2%)	22/128 (17.2%)	13/128 (10.2%)
<i>P</i> value	0.49	0.08	0.35
Class II			
–ve	44/194 (22.7%)	49/209 (23.4%)	30/209 (14.4%)
+ve	7/63 (11.1%)	8/65 (12.3%)	4/65 (6.2%)
<i>P</i> value	0.05	0.05	0.08

Among nonatopic children, there was a clear relationship between birth order and the presence of maternal anti-HLA antibodies. Furthermore, we observed that maternal anti-HLA class II (but not class I) antibodies were associated with significantly less atopy ($P = 0.05$) and less seasonal rhinitis ($P = 0.05$) in the offspring at age eight.

Our prevalence rates for anti-HLA antibodies were higher than in older studies, probably reflecting the higher sensitivity in our detection method (17). We did not HLA type the children in this study, and have made the assumption that maternal production of anti-HLA antibodies is in response to paternal antigens of foetal origin; a reasonable assumption because it is known that HLA antibodies are rare in nulliparous women (18) but become more prevalent with increasing parity (19, 20). Anti-HLA antibodies are more often detected where there is maternal–paternal HLA incompatibility (21–23) but their production reflects not only this but also access of paternal antigens to, and recognition by, the mother. Immune recognition by the mother will be influenced by a number of factors including her T cell repertoire and immunological status.

Previous studies have demonstrated that both HLA disparity and the maternal immune response to paternal

HLA antigens can alter the cytokine milieu between the mother and foetus (7). HLA class II but not class I antibodies were found to produce a stronger Th1 (IFN- γ) cytokine response in mixed lymphocyte reactions from the mother in response to foetal antigens (9). Other studies have shown that IFN- γ is critical in mediating tolerance in the offspring of mice (24, 25), a mechanism which may explain the reduced risk of atopy – and to a lesser extent seasonal wheeze – we observed in the offspring of mothers who had detectable levels of anti-HLA class II (but not class I) antibodies.

In a recent study (8), allergic women were reported to produce significantly lower IFN- γ (Th1) and IL-6 responses to HLA class II mismatch in mixed lymphocyte reactions, thus increasing, perhaps, the risk of allergic responses in their offspring. Here, we observed that allergic women respond differently to HLA, with a lower prevalence of anti-HLA class II antibodies compared with nonallergic women. These two observations are interesting; further work is required to understand whether the mechanisms involved as a result of HLA mismatch are the same for both HLA antibody and cytokine responses.

Previous studies have observed that atopic mothers have fewer number of offspring compared with nonatopic mothers (4, 5) and a higher number of pregnancies may be associated with loss of atopy in the mother (6). In our study, we found similar linear trends between proportions of mothers with detectable anti-HLA class I and II with both birth order and number of pregnancies irrespective of the mothers' atopic status.

A much stronger association between anti-HLA antibodies and birth order was observed in our birth cohort when the offspring were nonatopic; however, the less significant findings with atopic children may be due to a smaller number of atopic children (atopic children $n = 57$ compared with nonatopic children $n = 274$). Because patterns of maternal TH1/Th2 responses significantly correlate with foetal responses (8), we suggest that nonatopic offspring are exposed to a predominantly Th1 cytokine milieu during pregnancy, likely to have been provided by nonallergic mothers, who produce higher levels of IFN- γ (8). Again this speculation requires verification through further study.

The production of HLA antibodies reflects HLA disparity which itself has only recently been recognized as a significant determinant of allergic outcomes in offspring. It may be important in other diseases too. HLA disparity has been associated with improvement during pregnancy of symptoms in both inflammatory bowel disease and rheumatoid arthritis (26, 27); and a lack of HLA disparity has been reported to be a risk factor for the development of pre-eclampsia (28). The incidence of this last condition falls with increasing parity until the mother conceives with a new partner, when the risk returns to the equivalent of a first pregnancy (29), suggesting some form of tolerance to paternal HLA molecules with parity. Pre-eclamptic women have lower levels of paternal-induced IL-10 and higher levels of pro-inflammatory IL-6 suggesting either a lower number or functional activity of T regulatory cells, which downregulate both TH1 and Th2 responses (30). Interestingly, similar observations have also been shown with type 1 diabetes (31) and Crohn's disease (32).

There are several limitations to our study. We did not set out to examine parity-related changes in mothers and the maternal blood samples were collected 4 years after birth. We were unable to examine the effect of repeated pregnancies in the same mother, which may be an important aspect to examine in further longitudinal studies. We did not have data available on any blood transfusion in mothers which may induce anti-HLA antibodies. We have used HLA antibodies as a marker of HLA disparity but the production of anti-HLA antibodies themselves may have important immunomodulatory effects. It is interesting that antibodies to HLA class II antigens were more strongly associated with protection from atopy; MHC class II antigens are uniquely present on antigen presenting cells such as dendritic cells, raising the possibility that maternal antibodies to foetal MHC class II antigens may be modulating expression of HLA class II molecules, resulting in downregulation of antigen presentation and a more tolerogenic or anti-inflammatory phenotype of dendritic cell in the foetus. Both maternal and cord serum had been stored at -20°C for 8 years before our antibody measurements possibly reducing the sensitivity of the anti-HLA assay; because we detected a higher prevalence of anti-HLA antibodies than in previous studies, this is unlikely to have had a major effect and almost certainly introduced no bias. The definition of atopy in mother and child is restricted to three common airborne allergens (house dust mite, grass pollen and cat) and excludes sensitization to other allergens to which they may be exposed.

Our findings provide evidence in support of the hypothesis that the 'birth order' effect in children may at least in part be due to parity-related changes in the maternal immune response. This is the first study to observe an association between the presence of maternal anti-HLA class II antibodies with both birth order and protection from allergy in the offspring. Importantly, these observations are aligned to previous work demonstrating changes in cytokine milieu. Our results demonstrated that an increasing number of pregnancies was related to higher maternal anti-HLA class I and II antibodies that may be explained by a greater immunetolerance, which leads to a shift in the cytokine milieu towards a more balanced Th1:Th2 profile, in both mother and foetus, and an attenuation of allergy respiratory disease in the offspring.

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Author contributions

M Jones acquired and interpreted data, wrote the manuscript and was involved in the conception and design of the study. H Jeal was involved in the conception and design of the study and writing the manuscript. JM Harris analysed the

data and was involved with design of the study and writing the manuscript. JD Smith acquired and interpreted data. ML Rose critically revised the manuscript. A Newman Taylor conceived and designed the study. P Cullinan conceived and designed the study and critically reviewed the manuscript.

Capsule summary

Production of anti-HLA class II antibodies, as a result of parity-related changes in the maternal immune response, is associated with both birth order effect and protection from allergy in the offspring.

Key messages

- Maternal anti-HLA class II antibodies are associated with both birth order and protection from allergy in the offspring, particularly in the nonatopic children.
- 'Birth order' effect in children may at least in part be due to parity-related changes in the maternal immune response.

Conflict of interest

All authors declare that there are no conflicts of interest.

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