

BRIEF COMMUNICATION

HLA-DR polymorphism modulates response to house dust mites in a transgenic mouse model of airway inflammation

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Abstract

We and others have reported that HLA-DRB1*03 is associated with childhood asthma. To extend this observation and to prove this association, we sensitized and challenged either HLA-DR2 (HLA-DRB1*1502) or HLA-DR3 (HLA-DRB1*0301) transgenic mice with house-dust mite extract. Inflammatory cell counts and cytokine levels in the bronchoalveolar lavage (BAL) fluid between HLA-DR3 and DR2 mice were compared. HLA-DR3 transgenic mice had significantly elevated eosinophil counts, Interleukin-4 and Interleukin-13 levels in the BAL fluid but not interferon gamma- γ . Thus, our study suggests that HLA-DRB1*0301 plays an important role in mounting a Th2-predominant immune response to house dust mite and Th2-type inflammation in the lung.

Human leukocyte antigen (HLA) class II genes are an inheritable genetic factor, which may play an important role in the immunogenetic mechanisms underlying allergic asthma (1–6). Previous studies showed that HLA-D-restricted immunoglobulin E response to a short ragweed allergen was found among Caucasians carrying HLA-DR genes (7, 8) and HLA-D restriction element was the DR molecule (e.g. DRB1*03) (9, 10). Also, HLA-DR-restricted (e.g. DRB1*03) T-cell responses to house-dust mite (HDM) antigens have been reported (10–12). In a mouse model expressing HLA-DQ genes, when mice were sensitized with cockroach allergen through their airways, the airway inflammation incurred was similar to that seen in asthmatic patients with cockroach allergy (13, 14). Thus, HLA-restricted allergen-specific IgE or IgG response may be a plausible mechanism for childhood asthma given its association with a family history (15–19) and atopic sensitization (20).

Recently, we reported an association between HLA-DR3 allele and an increased risk of childhood asthma among Caucasian children of whom HLA-DR3 allele frequency was

11.4% (2). The literature suggests that HLA-DR3 gene frequencies were 10.6% in Caucasian, 11.5% in African Americans, and 3.6% in Orientals (21). Involvement of HLA-DR genes in asthma or its related phenotype has been supported by other studies (1, 22–28). For example, a recent genome-wide association study (GWAS) showed an association between HLA-DR and -DQ loci and asthma (26). However, other studies dispute the association (29–32). Apart from insufficient statistical power to address multiple comparisons, this inconsistency in the literature may be due to inadequate control of potential confounders or covariates in identifying HLA genes associated with childhood asthma or related phenotypes and population admixture. Controlling all pertinent covariates and population admixture is difficult to accomplish in an epidemiologic study. In addition, the specific immunogenetic mechanisms underlying the association between HLA-DR genes (e.g. DR3) and asthma have not been elucidated. Specifically, although a recent GWAS showed the association between HLA-DR genes and asthma, specific polymorphism within HLA-DR genes involved with the risk of asthma is

unknown (26). Thus, these limitations of previous studies and lack of information prevent us from advancing our understanding of the role of specific HLA class II genes such as DR3 in the etiology of childhood asthma.

To address the limitations of previous studies and to identify the potential immunogenetic mechanisms for the association between HLA-DR3 gene and asthma, we conducted a humanized transgenic mouse study that assessed the role of HLA-DR polymorphism in inflammatory responses to HDM in the lung. The HLA-DR3 and HLA-DR2 transgenic mice used in this study have been extensively characterized and widely used (33, 34). It has been shown earlier that the expressions of transgenic HLA class II molecules are comparable between these two strains. However, functional differences between HLA-DR2 and HLA-DR3 molecules elicit different disease phenotypes in several disease models such as experimental autoimmune thyroiditis, collagen-induced arthritis, and experimental allergic aspergillosis (35–37). However, these transgenic mice do not express any endogenous major histocompatibility complex (MHC) class II molecules as both the I-A and the I-E genes have been inactivated (AE^0) (38). Therefore, the MHC class II-restricted T-cell responses are exclusively restricted to the transgenically expressed HLA class II molecules.

Inflammatory responses in lungs of humanized transgenic mouse carrying HLA-DR3 gene

As a first step, we assessed whether humanized transgenic mice carrying HLA-DR3 elicited HDM-induced airway inflammation. House-dust mite extract (HDME) was obtained from Greer (Lenoir, NC). The results are summarized in Figure 1. The results showed that only those HLA-DR3 transgenic mice that were first subjected to subcutaneous sensitization followed by intranasal challenge with HDME had a significant number of eosinophils and macrophages in their bronchoalveolar lavage (BAL). On the contrary, HLA-DR3 transgenic mice that were subjected to either subcutaneous sensitization with HDME only or intranasal challenge with HDME only had little or no such inflammatory changes. These results suggest that humanized transgenic mice carrying HLA-DR3 gene were able to elicit an inflammatory response in airways through antigen-specific T-cell memory response to HDM.

HLA-DR polymorphism and inflammatory responses to HDM in lung

As a next step, we assessed the role of HLA-DR3 gene in lung inflammation in response to sensitization and intranasal challenge with HDM, compared with a control HLA-DR gene that was not associated with asthma according to the literature and our previous study and met the selection criteria. The control HLA class II gene was HLA-DR2. The comparisons of inflammatory cells and cytokines in BAL between transgenic

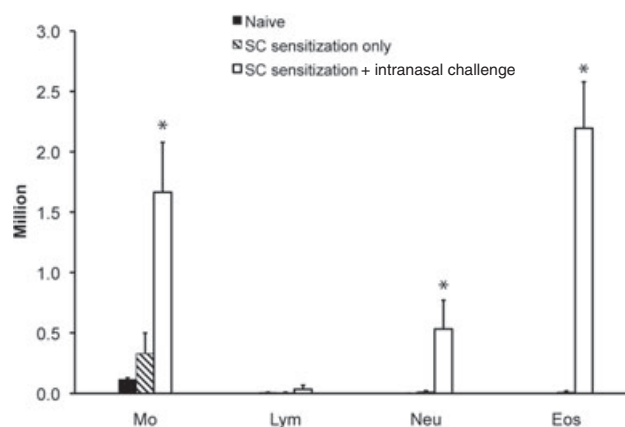


Figure 1 Both sensitization and challenge with house dust mite (HDM) are required to elicit an eosinophilic response in HLA-DR3 transgenic mice. Age-matched HLA-DR3 transgenic mice expressing functional HLA-DRA1*0101 and HLA-DRB1*0301 transgenes on the complete mouse major histocompatibility complex (MHC) class II-deficient were either subcutaneously sensitized with HDM alone or first sensitized followed by intranasal challenge with HDM (i.e. s.c. + intranasal). Sensitization was performed by shaving off fur on the back of mice under anesthesia and followed by subcutaneous injection of 100 μ g of house-dust mite extract (HDME) on days 0, 7, and 14. On days 21 and 22, mice were challenged intranasally with 100 μ g of the HDME. Mice were killed 2 days after intranasal challenge and the lungs were lavaged with 1 ml of phosphate-buffered saline (PBS) by cannulating the trachea with a blunt needle. The bronchoalveolar lavage (BAL) fluid was spun, the cell pellets were resuspended in 0.5 ml of PBS, counted, and spun onto glass slides. Cytospin preparations were stained with Giemsa stain and the differential counts obtained. Each bar represents mean $\pm$ SD from five mice. * $P < 0.05$ when compared with naïve and s.c.–intranasal groups (i.e. subcutaneous sensitization with HDM alone or without intranasal challenge with HDM) (Student's *t*-test using GRAPHPAD PRISM 5.0 software).

mice carrying HLA-DR3 and those carrying HLA-DR2 are summarized in Figures 2 and 3, respectively. The HLA-DR3 transgenic mice mounted a much stronger Th2-type inflammatory response to HDME when compared with the HLA-DR2 transgenic mice. As shown in Figure 2, only the HLA-DR3 transgenic mice both sensitized and challenged with HDME had very high total eosinophil count (1.8 ± 0.4 million) when compared with the HLA-DR3 transgenic mice which were only intranasally challenged with HDME without subcutaneous sensitization (0.006 ± 0.003 million). These results indicate that the eosinophilic response was specific and occurred only upon sensitization to HDME. On the other hand, the HLA-DR2 transgenic mice, which were sensitized and challenged with HDME, had very low eosinophil count in the BAL fluid (0.108 ± 0.036). Total macrophage, lymphocyte, and neutrophil counts in BAL were also elevated in the HLA-DR3 mice (1.56 ± 0.3 , 0.074 ± 0.02 , and 0.60 ± 0.14 million, respectively) when compared with the HLA-DR2 mice sensitized and challenged with HDME (0.19 ± 0.05 , 0.008 ± 0.0004 , and 0.09 ± 0.05 million, respectively).

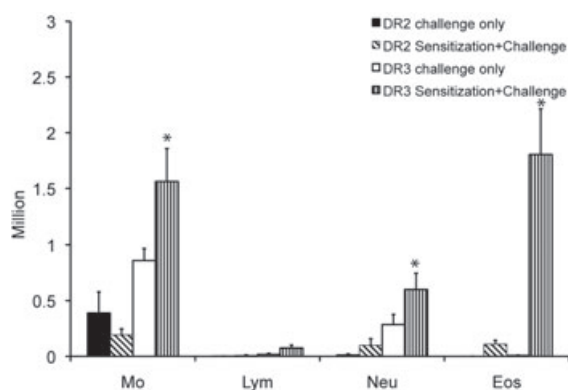


Figure 2 HLA-DR polymorphism determines the nature of inflammatory response elicited by house dust mite (HDM). Age-matched HLA-DR2 (expressing functional HLA-DRA1*0101 and HLA-DRB1*1502 transgenes) and HLA-DR3 transgenic mice were subcutaneously sensitized with HDM alone or first sensitized followed by intranasal challenge with HDM as described in Figure 1. Bronchoalveolar lavage (BAL) fluids were centrifuged and supernatants were stored frozen. Cell pellets were resuspended and cell counts in BAL fluids were determined. Each bar represents mean $\pm$ SD from five mice. * $P < 0.05$ when compared with similarly treated HLA-DR2 transgenic mice.

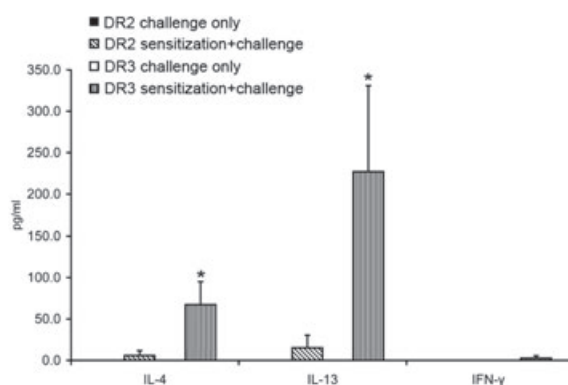


Figure 3 HLA-DR polymorphism determines the nature of cytokine response elicited by house dust mite (HDM). Age-matched HLA-DR2 and HLA-DR3 transgenic mice were subcutaneously sensitized with HDM alone or first sensitized followed by intranasal challenge with HDM as described in Figure 1. Concentrations of indicated cytokines in the bronchoalveolar lavage (BAL) fluids were determined by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) as per manufacturer's protocol. Each bar represents mean $\pm$ SD from five mice. * $P < 0.05$ when compared with similarly treated HLA-DR2 transgenic mice.

We next measured the levels of representative Th2- (IL-4 and IL-13) and Th1 (IFN- γ)-type cytokines in the BAL fluid. As shown in Figure 3, in concordance with the BAL cellularity, the HLA-DR3 transgenic mice sensitized and challenged with HDME had significantly higher levels of Th2 cytokines, IL-4 (67.30 ± 27.81 pg/ml) and IL-13 (227.0 ± 103 pg/ml) when compared with the HLA-DR2 transgenic mice (IL-4, 5.860 ± 5 pg/ml; IL-13, 15.20 ± 15.20 pg/ml).

Levels of IFN- γ were negligible in all groups. Taken together, the HLA-DR3 transgenic mice mounted a strong Th2 response to HDME when compared with HLA-DR2 transgenic mice.

These findings based on a transgenic mouse model confirm our previous findings from our epidemiologic study that HLA-DR3 was significantly associated with the risk of childhood asthma (2). HLA association studies in humans are complicated because of expression of multiple HLA class I and II alleles as haplotypes, linkage disequilibrium, and multiple confounding factors or covariate imbalance between comparison groups. Availability of transgenic mice expressing a given HLA class I or II allele overcomes these drawbacks. Because in our transgenic mouse model the endogenous MHC genes were completely removed and only specific human HLA-DR genes of interest were inserted and studied in controlled environment, our transgenic mouse model may be ideal to study the role of a specific HLA-DR polymorphism in inflammatory and immune responses to HDM. Nevertheless, the common disadvantages associated with transgenic technology such as inability to control the sites of transgene integration, copy number variations between different transgenes, and so on should be taken into consideration. Overall, the results of this study support the findings that HLA-DR polymorphism may be an important genetic factor for the development of asthma. Differential presentation of HDME epitopes by HLA-DR3 and HLA-DR2 might be a plausible reason for this differential response.

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