Published in final edited form as:

HLA. 2016 July ; 88(1-2): 25–34. doi:10.1111/tan.12851.

Influence of HLA-DR polymorphism and allergic sensitization on humoral immune responses to intact *Pneumococcus* in a transgenic mouse model

Youn H. Sheen^{1,2}, Govindarajan Rajagopalan³, Clifford M. Snapper⁴, Hirohito Kita⁵, Chung-il Wi¹, Puja J. Umaretiya^{6,7}, and Young J. Juhn^{1,5,#}

¹Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, Minnesota;

²Department of Pediatrics, CHA Gangnam Medical Center, CHA University School of Medicine, Seoul, Korea; ³Department of Immunology, Mayo Clinic, Rochester, Minnesota; ⁴Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland;

⁵Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota; ⁶Mayo Medical School, Rochester, Minnesota; ⁷Boston Children's Hospital, Boston, Massachusetts; USA.

Abstract

Asthma is independently associated with HLA-DR3 and increased risks of pneumococcal diseases. We aimed to determine whether HLA-DR polymorphism (HLA-DRB1*03), sensitization to house-dust mite (HDM), or their interaction affects humoral immune responses to pneumococcal polysaccharide and protein antigens of intact pneumococci. Induction of serum titers of anti-pneumococcal polysaccharide and anti-surface protein IgM and IgG in response to immunization with intact pneumococci (Pn) serotype 14 was determined using humanized HLA-DR3 and DR2 transgenic mice. Transgenic mice were sensitized by injecting HDM and challenged with intranasal HDM. Mice were subsequently immunized with heat-killed Pn14 at day 24. Serum titers of anti-phosphorylcholine (PC) IgM and IgG, anti-pneumococcal polysaccharide, capsular type 14 (PPS14) IgM and IgG, and anti-pneumococcal surface protein A (PspA) IgG were measured. We included a total of 44 mice (22 DR3 and 22 DR2 mice) and half of mice in each group were sensitized with HDM (ie, 22 HDM-sensitized and 22 control mice). HDM-sensitized mice, irrespective of HLA-DR polymorphism, had significantly lower humoral immune responses. HLA-DR3 mice, irrespective of HDM sensitization, elicited a significantly lower anti-PC IgG response. In contrast, the anti-PspA IgG response was higher in DR3 relative to DR2 mice. The effect of HDM sensitization on lowering humoral immune responses to Pn14 was observed in DR3 mice regardless of the nature of the antigen, whereas such decreases were

Corresponding author, Young J. Juhn, MD, MPH, Professor of Pediatrics, Department of Pediatric and Adolescent Medicine/Internal Medicine, Mayo Clinic, Rochester, Minnesota, USA, 200 1st Street SW, Rochester, MN 55905, Juhn.young@mayo.edu, Phone: 507-538-1642, Fax: 507-284-9744.

Conflict of interest

The authors have no conflict of interest to disclose.

Conflict of interest

Dr. Juhn is the PI of the Innovative Methods to Improve Asthma Disease Management Award supported from Genentech which has no relationship with the work presented in this manuscript. Otherwise, the study investigators have nothing to disclose that poses a conflict of interest.

observed only for the anti-PPS14 IgG and anti-PC IgM responses in DR2 mice. HDM sensitization lowered humoral immune responses to intact pneumococcus and this effect was significantly modified by the HLA-DR polymorphism.

Keywords

Adaptive Immunity; HLA-DR3; Sensitization; *Streptococcus pneumoniae*; Transgenic mice

Introduction

Streptococcus pneumoniae (hereafter termed *S. pneumoniae*) continues to be a significant cause of morbidity and mortality in both the US and worldwide (1, 2). It is reported that 43,500 cases and 5,000 deaths among subjects across all ages were caused by *S. pneumoniae* in the US in 2009 (3). The case fatality rate was estimated at 10% for all reported cases, increasing from 1.4% among subjects < 2 yr to 20.6% among subjects ≥ 80 yr (4, 5).

Accumulating evidence reports that asthmatic patients are at a significantly higher risk of developing invasive pneumococcal disease (IPD) (6–8). In this regard, the Advisory Committee on Immunization Practices (ACIP) updated their guideline and now recommends that all asthmatic adults receive a single dose of 23-valent pneumococcal polysaccharide vaccine (PPV-23) for the prevention of IPD (9).

While impaired innate immunity might play a role in the increased risk of developing serious pneumococcal infections (10), suboptimal adaptive immune functions have also been suggested to play a role in individuals with asthma (6). For instance, our recent results showed that only 7 of the 16 subjects with asthma (44%), compared to 12 of 14 control subjects (86%), had 12 or more positive serotype-specific polysaccharide antibodies (11). Other researchers have demonstrated suboptimal antibody responses to pneumococcal vaccines in children with asthma (12) and those with atopic dermatitis (13), suggesting that atopic individuals with Th2-predominant immune responses tend to be associated with poorer pneumococcal immune responses in humans (11, 14) and mice (15); however, the underlying mechanisms remain unknown. Given the association of certain genetic factors (e.g., HLA-DR) with the risk of asthma (16), allergic sensitization (17), and suboptimal humoral immune responses (18), it is worth investigating the role of HLA-DR genes (i.e., DR3), allergic sensitization, and their interactions in humoral immune responses to pneumococci. Specifically, our previous study showed that HLA-DR3 was associated with an increased risk of asthma, whereas HLA-DR2 was not (16).

To address this association, we conducted a study to assess the role of HLA-DR polymorphism (DR3 [HLA-DRB1*0301] vs. DR2 [HLA-DRB1*1502]), allergic sensitization (house dust mite [HDM]), and their interaction in the humoral immune response to pneumococcal polysaccharide and protein antigens of intact pneumococci using HLA-DR transgenic mice. We hypothesized that the HLA-DR3 gene and HDM sensitization interact with each other to result in a weaker antibody response to pneumococcal capsular polysaccharide, the phosphorylcholine component of C-polysaccharide, and pneumococcal surface protein antigens of intact pneumococci.

Materials and Methods

Study animals

We utilized 22 HLA-DR3 (HLA-DRB1*0301) transgenic mice and 22 HLA-DR2 (HLA-DRB1*1502) transgenic mice to compare anti-pneumococcal polysaccharide and anti-surface protein levels in response to intact pneumococci (serotype 14, Pn14). Pn 14 was selected for the challenge because this serotype is a representative serotype conferring high invasive disease potential (19), is one of the most common etiologic agents of pneumonia in adults (20) and of otitis media in children (21), and is known to have less virulence compared with other serotypes (eg, serotype 3), thus increasing the survival of the mice (22). The HLA-DR3 and HLA-DR2 transgenic mice used in this study have been extensively characterized and widely used in research (17, 23, 24). It has been demonstrated that the expressions of transgenic HLA class II molecules are comparable between these two strains; however, functional discrepancies between HLA-DR3 and HLA-DR2 molecules yield different disease phenotypes in several disease models such as experimental autoimmune thyroiditis (23), collagen-induced arthritis (25), experimental allergic aspergillosis (26), and allergic inflammation (17). These transgenic mice lack all endogenous conventional mouse major histocompatibility complex (MHC) class II molecules as both the I-A and the I-E genes have been inactivated (AE^0) (27). Accordingly, the MHC class II-restricted T-cell responses are exclusively attributed to the transgenically expressed HLA class II molecules. House dust mite extract (HDME) was obtained from Greer (Lenoir, NC). All animal experiments in this study were conducted in accordance with the principles set forth in the Guide for Care and Use of Laboratory Animals (28) and approved by the IACUC of the Mayo Clinic.

Study design

Mice were sensitized to HDME by injecting 100 μ g of HDME subcutaneously on days 0, 7, and 14. They were challenged with 100 μ g of HDM intranasally on days 21 and 22 under anesthesia. Mice were subsequently immunized with heat-killed Pn14 (5×10^8 C.F.U. equivalents/mouse) intraperitoneally on day 24 and given a booster immunization on day 38 (Figure 1).

Data and sample collection

A pre-immunization bleed was performed on day 24 (T0) and repeated on day 38 (14 days post-primary immunization, T1). Serum titers of IgM and/or IgG for the following antigen specificities were measured by ELISA in order to assess immune responses to different pneumococcal antigens: PPS14, phosphorylcholine (PC), and determinant of C-polysaccharide pneumococcal surface protein A (PspA). PPS14 uniquely demonstrates very high immunogenicity and specificity when used in ELISA assays to measure serum titers of anti-PPS14 (29). PC is a haptenic component of teichoic acid, expressed in the pneumococcal cell wall, and of lipoteichoic acid, expressed in the pneumococcal cell membrane. PspA, a choline-binding protein, is a highly immunogenic pneumococcal cell wall protein (30). The IgM anti-PPS14 and PC responses are T cell-independent, whereas the IgG anti-PPS14, PC, and PspA responses are dependent on CD4⁺ T cells. Nevertheless, the immunologic parameters underlying the IgG anti-PPS14 and PC responses (i.e.

polysaccharides) differ from those for the IgG anti-PspA response (i.e. protein) (29). The median change of antibody titers (i.e., fold-increases from T0 to T1) was used for comparison.

Measurement of serum titers of Ag-specific IgG and IgM by ELISA

Serum titers of anti-PPS14 IgG and IgM, anti-PC IgG and IgM, and anti-PspA IgG were measured by ELISA. Specifically, plates were coated with 5 µg/mL PPS14, PC–keyhole limpet hemocyanin, or PspA (100 µL/well) followed by 5-fold dilutions of serum samples, starting at a 1/100 serum dilution. For the PPS14-specific ELISA, no cell wall polysaccharide was added to serum samples for blocking anti-PC antibodies, because PPS14-coated plates do not bind any detectable PC-specific IgG or IgM. Alkaline phosphatase–conjugated polyclonal goat anti-mouse IgM or IgG was then added at a final concentration of 1 µg/mL (50 µL/well), followed by substrate (p-nitrophenyl phosphate, disodium) at 1 mg/mL for color development. Color was read at an absorbance of 405 nm on a Multiskan Ascent ELISA reader (Thermo Labsystems, Helsinki, Finland).

Statistical analysis

Data were expressed as median [interquartile range] of the individual serum samples. Mann–Whitney *U* test was used to determine any differences in antibody titers between groups. Analyses were performed by using JMP 10 (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided and statistical significance was determined at a *P*-value of <0.05.

Results

We included a total of 44 mice (22 DR3 mice and 22 DR2 mice) and half of mice in each group were sensitized with HDM (i.e., 22 HDM-sensitized mice and 22 non-sensitized mice).

Comparison of various antibody responses to intact, heat-killed pneumococcus, capsular serotype 14 (Pn14) between HDM-sensitized and non-sensitized mice irrespective of HLA-DR polymorphism

The results are depicted in Figure 2. Overall, HDM-sensitized mice showed significantly lower antibody responses to all pneumococcal antigens, compared to non-sensitized mice. Specifically, the relative fold-increases in serum titers between HDM non-sensitized and HDM-sensitized mice, respectively, were as follows: anti-PPS14 IgG (3.0 [1.6, 12.6] vs. 0.53 [0.35, 0.92], *P*<0.001), anti-PPS14 IgM (5.5 [1.7, 49.7] vs. 1.6 [0.1, 8.3], *P*=0.01), anti-PC IgG (39.5 [10.0, 240.1] vs. 12.3 [1.4, 45.0], *P*=0.008), anti-PC IgM (29.6 [2.0, 98.4] vs. 0.87 [0.31, 19.1], *P*=0.002), and anti-PspA IgG (92.6 [40.1, 173.5] vs. 36.5 [9.6, 70.9], *P*=0.049) compared to non-sensitized mice.

Comparison of various antibody responses to intact, heat-killed Pn14 between HLA-DR2 and HLA-DR3 mice, irrespective of sensitization to HDM

The results are summarized in Figure 3. HLA-DR3 mice mounted a significantly lower anti-PC IgG response (74.5 [13.3, 240.1] vs. 8.7 [1.4, 38.5], *P*=0.002) and a significantly higher

anti-PspA IgG response (40.0 [8.0, 60.2] vs. 122.1 [20.3, 179.6], $P=0.01$) as compared to HLA-DR2 mice. However, fold-increases in the serum titers for anti-PPS14 IgG (1.2 [0.52, 2.0] vs. 1.8 [0.48, 10.7], $P=0.22$), anti-PPS14 IgM (5.5 [1.6, 22.7] vs. 1.6 [0.96, 17.5], $P=0.19$), and anti-PC IgM (16.5 [0.76, 115.5] vs. 2.1 [0.83, 36.9], $P=0.21$) did not significantly differ between HLA-DR2 and HLA-DR3 mice.

The effect of sensitization to HDM on the basis of HLA-DR polymorphism in the humoral immune response to Pn14

Figure 4 and 5 show the effect modification of sensitization to HDM on humoral antibody immune responses according to HLA-DR polymorphism. In HLA-DR3 mice, HDM-sensitized mice mounted significantly lower immune responses with anti-PPS14 IgG (4.8 [2.3, 16.8] vs. 0.48 [0.36, 1.4], $P<0.001$), anti-PPS14 IgM (3.1 [1.2, 56.6] vs. 1.2 [0.07, 2.3], $P=0.01$), anti-PC IgG (27.1 [11.2, 40.9] vs. 1.4 [0.39, 3.7], $P=0.01$), anti-PC IgM (11.8 [1.5, 49.0] vs. 0.90 [0.31, 12.6], $P=0.02$), and anti-PspA IgG (172.8 [95.9, 239.5] vs. 34.7 [10.5, 149.3], $P=0.02$), respectively, when compared to non-sensitized mice (Figure 4).

In HLA-DR2 mice, while sensitized mice mounted less robust immune responses with anti-PPS14 IgG (1.9 [1.4, 4.4] vs. 0.58 [0.31, 0.85], $P<0.001$) and anti-PC IgM (83.3 [2.8, 133.0] vs. 0.83 [0.31, 51.2], $P=0.02$), levels for anti-PPS14 IgM (5.8 [2.9, 39.3] vs. 4.5 [0.11, 20.3], $P=0.32$), anti-PC IgG (233.0 [5.0, 301.2] vs. 28.6 [14.3, 77.6], $P=0.056$), and anti-PspA IgG (50.2 [4.6, 92.2] vs. 38.2 [8.9, 43.6], $P=0.39$) did not significantly differ between sensitized and non-sensitized mice (Figure 5).

Discussion

The aim of this study was to determine whether HLA-DR3 gene, sensitization to HDM, or their interactions contribute to a differential humoral immune response to pneumococcal capsular polysaccharide, the phosphorylcholine component of C-polysaccharide, and pneumococcal surface protein antigens of intact pneumococci. While HDM sensitization consistently suppressed humoral immune responses to Pn14 regardless of type of antigens, HLA-DR polymorphism modified such effects of HDM sensitization on antibody production. To the best of our knowledge, this is the first murine study to determine the effect of the underlying HLA-DR polymorphism, allergic sensitization (i.e., HDM), and their interactions on anti-pneumococcal antibody responses.

Previous studies have suggested that patients with asthma are at increased risk of developing IPD (7, 8, 11, 31). A recent study of an adult population reported that the risk of IPD in patients with asthma is double that of healthy subjects (31). Another study assessing 174 cases of IPD in a retrospective chart review of 3,941 patients prior to the introduction of pneumococcal vaccines showed that IPD was closely correlated with a past history of asthma (7). Our recent study looking at fold-increases in antibody titers between pre- and post-vaccination with PPV-23 in relation to asthma found that subjects with a less robust immune response to PPV-23 were more likely to have asthma (Y. H. Sheen, Y. Juhn, S. Kizilbash, E. Ryoo, C. I. Wi, M. Park, R. S. Abraham, E. Ryu, and R. Divekar, submitted for publication). These previous studies are in line with our current observation that HLA-DR3 transgenic mice sensitized to HDM demonstrated less robust immune responses to intact

pneumococcus compared to non-sensitized HLA-DR3 transgenic mice, which could subsequently result in serious pneumococcal diseases later in life.

Why HDM-sensitized HLA-DR3 mice mount less robust humoral immune responses to intact pneumococcus when compared with non-sensitized HLA-DR3 mice is unknown. One possible explanation is that sensitized subjects with asthma susceptibility genes may have defects in their adaptive immune system. Patients with asthma or atopic conditions have enhanced Th2 activity as reflected by Th2 cell predominance in the airways (4, 32), whereas healthy subjects do not have such Th2-predominant immune environment (33). Pneumococcal polysaccharide induces a T-cell-independent type II antibody response, indicating that T cells are not required to produce antibodies, but may still help the maturation of B-cells with regard to antibody production (34). Although antibody responses to purified PPS14 and phosphorylcholine component of C-polysaccharide are T-cell independent, the polysaccharide-specific IgG response to intact pneumococcus, namely, anti-PPS14 IgG and anti-PC IgG, as well as the anti-PspA IgG, require CD4⁺ T cell help for optimal antibody responses (29). In contrast, the anti-PPS14 IgM and anti-PC IgM responses are T cell-independent. A murine model investigating humoral immune responses to intact *S. pneumoniae* reported that Th2 cytokines decreased antibody responses to pneumococcal polysaccharide antigens, whereas Th1 cytokines enhanced antibody responses (15). Furthermore, a recent study assessing the immunogenicity of live attenuated yellow fever virus vaccination of atopic dermatitis subjects demonstrated that atopic dermatitis patients mounted weaker antiviral CD4⁺ and CD8⁺ T cell responses to vaccines when compared with non-atopic control subjects, suggesting that atopic conditions may have a suppressive influence on T cell immune responses to vaccines (35). Another study comparing serotype-specific antibody responses to pneumococcal polysaccharide antigens in subjects with and without asthma found that those with asthma had significantly lower serotype-specific pneumococcal antibody levels than those without asthma, suggesting that Th2 immune profiles of subjects with asthma may have a down-regulating effect on their serotype-specific antibody responses to pneumococcus (11). However, these previous studies are limited in that they did not analyze polymorphisms of asthma susceptibility genes in their subjects. We showed using a transgenic mouse model that HLA-DR polymorphisms and allergic sensitization interact with each other to down-regulate humoral immune responses to intact pneumococci. Another possible mechanism may be ascribed to the suboptimal function of HLA-DR3 Class II molecules in antigen presenting cells, such as dendritic cells, in presenting processed pneumococcal antigenic peptides. This may result in suboptimal CD4⁺ T cell activation and inadequate help to B-cells during the antibody maturation process, subsequently leading to ineffective or less productive humoral antibodies to foreign antigens (36). Moreover, altered CD4⁺ T cell repertoire in HLA-DR3 transgenic mice compared with HLA-DR2 transgenic mice due to differences in the presentation of peptides during thymic selection may also contribute to these differential responses between HLA-DR3 and HLA-DR2 transgenic mice. Collectively, we speculate that allergen-sensitized subjects with asthma susceptibility genes may have incompetent adaptive immunity, as shown by reduced humoral immune responses to intact pneumococcus, thereby leading to an increased risk of developing serious pneumococcal diseases.

The present study may have a few important implications. It may be important for clinicians to discern that patients with asthma may have suboptimal humoral immune responses to pneumococcus, especially those with allergic sensitization to HDM and a certain genetic background. The present results further suggest that patients with atopic asthma may have an increased risk of microbial infection due to suboptimal adaptive immunity as observed in our study. Therefore, our study results provide an important insight into the role of adaptive immunity in accounting for the relationship between asthma and the increased risk of pneumococcal diseases, given the disproportionate research effort and focus on innate immunity over the past decade. While we demonstrated in a previous study that patients with asthma had suboptimal cell-mediated immunity in response to measles, mumps, and rubella (37), we did not measure cell-mediated immunity in the present study, which needs to be addressed in future studies.

Of note, there were differential humoral immune responses between HLA-DR3 and DR2 mice when challenged by Pn 14 as depicted in Figure 3. Because these mice do not express any endogenous conventional mouse MHC class II molecules and these HLA polymorphisms previously showed different disease phenotypes (17, 23, 25, 26), these differential humoral immune responses are likely due to HLA polymorphisms playing a key role in eliciting differential humoral immune responses to intact pneumococci 14.

The main strength of the present study is that we used a humanized transgenic mouse model based on human HLA genes that do not express any endogenous MHC class II molecules (27). Another important strength is the use of intact pneumococcus to elicit humoral immune responses to various antigens. Finally, we used various antigens including pneumococcal polysaccharide and protein antigens for the study. Nevertheless, the present study has limitations. First, a relatively small number of mice were included in the study; however, the study was able to detect differential humoral immune responses by HLA DR polymorphism and by sensitization. Second, cellular immunological markers (eg, Th1 and Th2 cytokines) were not assessed to support our findings. However, we previously reported predominant Th2 immune responses (elevated IL-4 and increased eosinophil counts in BAL) in HLA-DR3 mice after HDM sensitization, but such Th2 immune responses were not observed in HLA-DR2 mice. Rather, they showed predominant Th1 immune responses (ie, IFN- γ secretion and not increased eosinophil counts in BAL) (17). Third, we did not determine functional phenotypes of asthma such as lung function testing in mice to see if they have reduced lung functions, a characteristic feature of asthma.

In conclusion, the present murine study revealed that HDM sensitization reduced humoral immune response to pneumococci when compared with those without sensitization and such effect on humoral immune response was modified by HLA-DR polymorphism. Further studies are warranted to confirm the clinical significance of our study findings of increased susceptibility to pneumococcal infections in HDM-sensitized subjects with HLA-DR polymorphism and to determine ways to boost humoral immune responses to pneumococcal antigens among HDM-sensitized subjects with asthma.

Acknowledgements

We would like to thank Mrs. Kelly Okeson for administrative assistance and support. This work was supported by the Scholarly Clinician Award of Mayo Foundation and the National Institute of Allergy and Infectious Diseases (R21 AI101277).

Abbreviations:

ACIP	Advisory Committee on Immunization Practices
Anti-PC IgG	Anti-phosphorylcholine IgG
Anti-PC IgM	Anti-phosphorylcholine IgM
Anti-PPS 14 IgG	Anti-pneumococcal polysaccharide, capsular type 14 IgG
Anti-PPS 14 IgM	Anti-pneumococcal polysaccharide, capsular type 14 IgM
Anti-PspA IgG	Anti-pneumococcal surface protein A IgG
HDM	House-dust mite
HDME	House-dust mite extract
IACUC	Institutional Animal Care and Use Committee
IPD	Invasive pneumococcal disease
Pn14	<i>Streptococcus pneumoniae</i> , capsular type 14
PPV-23	23-valent pneumococcal polysaccharide vaccine
<i>S. pneumoniae</i>	<i>Streptococcus pneumoniae</i>

References

- Tomczyk S, Bennett NM, Stoecker C, et al. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among adults aged ≥ 65 years: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. 2014;63:822–5. [PubMed: 25233284]
- Bogaert D, Engelen MN, Timmers-Reker AJ, et al. Pneumococcal carriage in children in The Netherlands: a molecular epidemiological study. Journal of clinical microbiology. 2001;39:3316–20. [PubMed: 11526169]
- 2012 CfDCaP. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, *Streptococcus pneumoniae*, 2010 Available from: <http://www.cdc.gov/abcs/reports-findings/survreports/spneu10pdf>.
- Robinson KA, Baughman W, Rothrock G, et al. Epidemiology of invasive *Streptococcus pneumoniae* infections in the United States, 1995–1998: Opportunities for prevention in the conjugate vaccine era. JAMA. 2001;285:1729–35. [PubMed: 11277827]
- Rock C, Sadlier C, Fitzgerald J, et al. Epidemiology of invasive pneumococcal disease and vaccine provision in a tertiary referral center. Eur J Clin Microbiol Infect Dis. 2013;32:1135–41. [PubMed: 23525795]
- Juhn YJ. Risks for infection in patients with asthma (or other atopic conditions): is asthma more than a chronic airway disease? J Allergy Clin Immunol. 2014;134:247–57; quiz 58–9. [PubMed: 25087224]

7. Juhn YJ, Kita H, Yawn BP, et al. Increased risk of serious pneumococcal disease in patients with asthma. *J Allergy Clin Immunol.* 2008;122:719–23. [PubMed: 18790525]
8. Talbot TR, Hartert TV, Mitchel E, et al. Asthma as a risk factor for invasive pneumococcal disease. *N Engl J Med.* 2005;352:2082–90. [PubMed: 15901861]
9. Daly TM, Hill HR. Use and Clinical Interpretation of Pneumococcal Antibody Measurements in the Evaluation of Humoral Immune Function. *Clin Vaccine Immunol.* 2015;22:148–52. [PubMed: 25520149]
10. Habibzay M, Saldana JJ, Goulding J, Lloyd CM, Hussell T. Altered regulation of Toll-like receptor responses impairs antibacterial immunity in the allergic lung. *Mucosal Immunol.* 2012;5:524–34. [PubMed: 22549744]
11. Jung JA, Kita H, Dhillon R, et al. Influence of asthma status on serotype-specific pneumococcal antibody levels. *Postgraduate medicine.* 2010;122:116–24. [PubMed: 20861595]
12. Lee HJ, Kang JH, Henrichsen J, et al. Immunogenicity and safety of a 23-valent pneumococcal polysaccharide vaccine in healthy children and in children at increased risk of pneumococcal infection. *Vaccine.* 1995;13:1533–8. [PubMed: 8578838]
13. Arkwright PD, Patel L, Moran A, Haeney MR, Ewing CI, David TJ. Atopic eczema is associated with delayed maturation of the antibody response to pneumococcal vaccine. *Clinical and experimental immunology.* 2000;122:16–9. [PubMed: 11012612]
14. Podjasek JC, Jung JA, Kita H, Park MA, Juhn YJ. The FACT score in predicting pneumococcal antibody levels in asthmatics. *J Asthma.* 2015;52:370–5. [PubMed: 25329680]
15. Khan AQ, Shen Y, Wu ZQ, Wynn TA, Snapper CM. Endogenous pro- and anti-inflammatory cytokines differentially regulate an in vivo humoral response to *Streptococcus pneumoniae*. *Infection and immunity.* 2002;70:749–61. [PubMed: 11796608]
16. Juhn YJ, Kita H, Lee LA, et al. Childhood asthma and human leukocyte antigen type. *Tissue antigens.* 2007;69:38–46. [PubMed: 17212706]
17. Rajagopalan G, Tilahun AY, Iijima K, David CS, Kita H, Juhn YJ. HLA-DR polymorphism modulates response to house dust mites in a transgenic mouse model of airway inflammation. *Tissue antigens.* 2011;77:589–92. [PubMed: 21447115]
18. Poland GA, Ovsyannikova IG, Jacobson RM, et al. Identification of an association between HLA class II alleles and low antibody levels after measles immunization. *Vaccine.* 2001;20:430–8. [PubMed: 11672906]
19. Sleeman KL, Griffiths D, Shackley F, et al. Capsular serotype-specific attack rates and duration of carriage of *Streptococcus pneumoniae* in a population of children. *J Infect Dis.* 2006;194:682–8. [PubMed: 16897668]
20. Bewick T, Sheppard C, Greenwood S, et al. Serotype prevalence in adults hospitalised with pneumococcal non-invasive community-acquired pneumonia. *Thorax.* 2012;67:540–5. [PubMed: 22374921]
21. Rodgers GL, Arguedas A, Cohen R, Dagan R. Global serotype distribution among *Streptococcus pneumoniae* isolates causing otitis media in children: potential implications for pneumococcal conjugate vaccines. *Vaccine.* 2009;27:3802–10. [PubMed: 19446378]
22. Xu JH, Dai WJ, Chen B, Fan XY. Mucosal immunization with PsaA protein, using chitosan as a delivery system, increases protection against acute otitis media and invasive infection by *Streptococcus pneumoniae*. *Scand J Immunol.* 2015;81:177–85. [PubMed: 25565478]
23. Kong YC, Lomo LC, Motte RW, et al. HLA-DRB1 polymorphism determines susceptibility to autoimmune thyroiditis in transgenic mice: definitive association with HLA-DRB1*0301 (DR3) gene. *J Exp Med.* 1996;184:1167–72. [PubMed: 9064334]
24. Mangalam AK, Rajagopalan G, Taneja V, David CS. HLA class II transgenic mice mimic human inflammatory diseases. *Advances in immunology.* 2008;97:65–147. [PubMed: 18501769]
25. Taneja V, Griffiths MM, Luthra H, David CS. Modulation of HLA-DQ-restricted collagen-induced arthritis by HLA-DRB1 polymorphism. *Int Immunol.* 1998;10:1449–57. [PubMed: 9796911]
26. Koehm S, Slavin RG, Hutcheson PS, Trejo T, David CS, Bellone CJ. HLA-DRB1 alleles control allergic bronchopulmonary aspergillosis-like pulmonary responses in humanized transgenic mice. *J Allergy Clin Immunol.* 2007;120:570–7. [PubMed: 17561243]

27. Cheng S, Smart M, Hanson J, David CS. Characterization of HLA DR2 and DQ8 transgenic mouse with a new engineered mouse class II deletion, which lacks all endogenous class II genes. *J Autoimmun.* 2003;21:195–9. [PubMed: 14599844]
28. Council NR. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academy Press, 1996.
29. Snapper CM. Mechanisms underlying in vivo polysaccharide-specific immunoglobulin responses to intact extracellular bacteria. *Annals of the New York Academy of Sciences.* 2012;1253:92–101. [PubMed: 22288494]
30. Briles DE, King JD, Gray MA, McDaniel LS, Swiatlo E, Benton KA. PspA, a protection-eliciting pneumococcal protein: immunogenicity of isolated native PspA in mice. *Vaccine.* 1996;14:858–67. [PubMed: 8843627]
31. Inghammar M, Engstrom G, Kahlmeter G, Ljungberg B, Lofdahl CG, Egesten A. Invasive pneumococcal disease in patients with an underlying pulmonary disorder. *Clin Microbiol Infect.* 2013;19:1148–54. [PubMed: 23464817]
32. James KM, Peebles RS, Jr., Hartert TV Response to infections in patients with asthma and atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol.* 2012;130:343–51. [PubMed: 22846746]
33. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med.* 1992;326:298–304. [PubMed: 1530827]
34. Mond JJ, Vos Q, Lees A, Snapper CM. T cell independent antigens. *Current opinion in immunology.* 1995;7:349–54. [PubMed: 7546399]
35. Slifka MK, Leung DY, Hammarlund E, et al. Transcutaneous yellow fever vaccination of subjects with or without atopic dermatitis. *J Allergy Clin Immunol.* 2014;133:439–47. [PubMed: 24331381]
36. Crotty S A brief history of T cell help to B cells. *Nat Rev Immunol.* 2015;15:185–9. [PubMed: 25677493]
37. Yoo KH, Agarwal K, Butterfield M, Jacobson RM, Poland GA, Juhn YJ. Assessment of humoral and cell-mediated immune response to measles-mumps-rubella vaccine viruses among patients with asthma. *Allergy and asthma proceedings : the official journal of regional and state allergy societies.* 2010;31:499–506.

Figure 1:

Experimental design for sensitization to house dust mite, immunization with *Streptococcus pneumoniae* protocol for the murine model, and measurement of humoral immune responses to intact pneumococcus serotype 14. HDM, house dust mite; s/c, subcutaneous sensitization; i/n, intranasal sensitization; i/p, intraperitoneal sensitization; Pn14: *Streptococcus pneumoniae*, capsular type 14.

Figure 2.

Comparison of various antibody responses as represented by fold-increases to pneumococcus serotype 14 according to sensitization to house-dust mite irrespective of HLA-DR types. Data are expressed as median [interquartile range] of the individual serum samples. (A) Anti-PPS14 IgG, (B) Anti-PPS14 IgM, (C) Anti-PC IgG, (D) Anti-PC IgM, and (E) Anti-PspA IgG. Fold-increases in antibody titers are calculated as antibody titer on day 38 (ie, 14 days post-immunization) divided by antibody titer on day 24 (ie, baseline). Anti-Pneumococcal polysaccharides 14 IgG, anti-PPS 14 IgG; anti-Pneumococcal polysaccharides 14 IgM, anti-PPS 14 IgM; anti-phosphorylcholine IgG, anti-PC IgG; anti-phosphorylcholine IgM, anti-PC IgM; anti-Pneumococcal surface protein A IgG, anti-PspA IgG.

Figure 3.

Comparison of various antibody responses as represented by fold-increases to pneumococcus serotype 14 according to HLA-DR polymorphisms. Data are expressed as median [interquartile range] of the individual serum samples. (A) Anti-PPS14 IgG, (B) Anti-PPS14 IgM, (C) Anti-PC IgG, (D) Anti-PC IgM, and (E) Anti-PspA IgG. Fold-increases in antibody titers are calculated as antibody titer on day 38 (ie, 14 days post-immunization) divided by antibody titer on day 24 (ie, baseline). Anti-Pneumococcal polysaccharides 14 IgG, anti-PPS 14 IgG; anti-Pneumococcal polysaccharides 14 IgM, anti-PPS 14 IgM; anti-phosphorylcholine IgG, anti-PC IgG; anti-phosphorylcholine IgM, anti-PC IgM; anti-Pneumococcal surface protein A IgG, anti-PspA IgG.

Figure 4.

Comparison of various antibody responses as represented by fold-increases to pneumococcus serotype 14 between non-sensitized and sensitized HLA-DR3 mice. Data are expressed as median [interquartile range] of the individual serum samples. (A) Anti-PPS14 IgG, (B) Anti-PPS14 IgM, (C) Anti-PC IgG, (D) Anti-PC IgM, and (E) Anti-PspA IgG. Fold-increases in antibody titers are calculated as antibody titer on day 38 (ie, 14 days post-immunization) divided by antibody titer on day 24 (ie, baseline). Anti-Pneumococcal polysaccharides 14 IgG, anti-PPS 14 IgG; anti-Pneumococcal polysaccharides 14 IgM, anti-PPS 14 IgM; anti-phosphorylcholine IgG, anti-PC IgG; anti-phosphorylcholine IgM, anti-PC IgM; anti-Pneumococcal surface protein A IgG, anti-PspA IgG.

Figure 5.

Comparison of various antibody responses as represented by fold-increases to pneumococcus serotype 14 between non-sensitized and sensitized HLA-DR2 mice. Data are expressed as median [interquartile range] of the individual serum samples. (A) Anti-PPS14 IgG, (B) Anti-PPS14 IgM, (C) Anti-PC IgG, (D) Anti-PC IgM, and (E) Anti-PspA IgG. Fold-increases in antibody titers are calculated as antibody titer on day 38 (ie, 14 days post-immunization) divided by antibody titer on day 24 (ie, baseline). Anti-Pneumococcal polysaccharides 14 IgG, anti-PPS 14 IgG; anti-Pneumococcal polysaccharides 14 IgM, anti-PPS 14 IgM; anti-phosphorylcholine IgG, anti-PC IgG; anti-phosphorylcholine IgM, anti-PC IgM; anti-Pneumococcal surface protein A IgG, anti-PspA IgG.