CONCISE COMMUNICATION

Associations between Human Leukocyte Antigens and Nonresponsiveness to Influenza Vaccine

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Influenza remains a major cause of morbidity and mortality, particularly in at-risk groups where vaccination reduces complications of infection but is not universally protective. In order to determine whether human leukocyte antigen (HLA) class II polymorphisms modulate anti-influenza antibody responses to vaccination, a cohort of HLA-typed at-risk donors was investigated. The subjects were recruited from a single urban family practice. Hemagglutination-inhibition (HAI) titers were measured immediately before and 28 days after subunit vaccination. Nonresponsiveness was defined as failure to mount an HAI response to any component of the trivalent influenza vaccine. When the nonresponders and responders with HLA class II were compared, the nonresponder group had more HLA-DRB1*07–positive donors (13/32 vs. 6/41 responders; $P = .016$, Fisher's exact test) and fewer HLA-DQB1*0603-9/14–positive donors (2/32 vs. 14/41 responders; $P = .0045$). Thus, polymorphisms in HLA class II molecules appear to modulate antibody responses to influenza vaccination.

Influenza remains a major cause of morbidity and mortality [1]. During a typical outbreak, 10%–20% of the population develop serologic evidence of infection [2], and, among at-risk groups, such as the elderly and those with chronic respiratory, cardiac, or metabolic diseases, the risk for hospitalization may approach 1 in 300 and the risk of death 1 in 1500 [2]. Vaccination remains the best defense against influenza [3]; it reduces the complications of infection, hospital admissions, and mortality during winter epidemic periods [3, 4]. Vaccines are poor at boosting cytotoxic T cell responses [5] and function primarily by inducing influenza-specific antibodies. The best protection is afforded by hemagglutination-inhibition (HAI) antibodies [4], which block the function of hemagglutinin, the viral surface-coat protein responsible for attachment to and entry into host cells. This protection is short lived because new virus strains regularly appear with mutations in neutralizing antibody binding sites (antigenic drift), necessitating the annual administration of influenza vaccines [3].

The clinical effectiveness of influenza vaccines depends on the immunocompetence of the recipient, previous exposure to influenza and influenza vaccines, and the closeness of match between the vaccine and circulating influenza strains [3, 4]. When the vaccine and circulating strain are well matched, protection rates can approach 90% among young, fit adults and 60% among the elderly [4].

Given the huge health burden caused by influenza and the ever present threat of a future pandemic, failure to mount an immune response is a significant public health issue. We therefore investigated nonresponsiveness to influenza vaccination, as assessed by HAI responses, in a cohort of at-risk persons who were recruited from a single urban family practice in the United Kingdom and for whom annual influenza vaccination would be recommended by the Advisory Committee on Immunization Practices (ACIP) [3].

Generation of anti-hemagglutinin antibodies by B lymphocytes is under the control of CD4 T cells [6]. Because CD4 T cells recognize antigens in association with HLA class II molecules [7], the primary aim of this study was to investigate associations between HLA class II molecules and nonresponsiveness to influenza vaccination.

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Informed consent was obtained from study subjects. The study was conducted within the human experimentation guidelines of East London and the City Health Authority local research ethics committee and the University of Wales College of Medicine.

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Methods

Subjects and vaccine. In total, 73 donors were recruited from a single urban family practice in Birmingham, United Kingdom. Donors were white and 34–83 years old (mean, 64.2 years). Donors matched the ACIP definitions of at risk [3]: 68% were ⩾65 years old, 41% had a cardiac condition (ischemic heart disease, heart failure, or dysrhythmia), 21% had diabetes mellitus, 26% had respiratory disease (asthma or chronic obstructive pulmonary disease), 16% had a miscellaneous at-risk condition (7% were immunocompromised, the majority were taking oral corticosteroids, and none were positive for human immunodeficiency virus), 7% had an endocrine disorder, and 3% had a chronic neurologic condition. Donors received trivalent influenza vaccine containing 15 μg of influenza A/Texas/36/91 (H1N1), 15 μg of influenza A/Resivir9 (H3N2), and 15 μg of influenza B/Harbin/7/94.

HAI titers. HAI of the donors against influenza A/Texas/36/91 (H1N1), A/Resivir9 (H3N2), and B/Harbin/7/94 was assessed immediately before and 28 days after vaccination. Antiserum samples were treated with receptor destroying enzyme (RDE; provided by the National Institute of Biological Standards and Control, South Mimms, UK), to destroy nonspecific inhibitors of hemagglutination. We incubated 50 μL of antiserum with 200 μL of RDE overnight at 37°C. The reaction was stopped by denaturing the enzyme at 56°C for 30 min. Hemagglutination was measured by the agglutination of 0.7% (vol/vol) turkey red blood cells (Advanced Protein Products) in 96-well V-bottomed microtiter plates (Greiner) with doubling dilutions of virus in 100 μL of PBS. HAI titers were determined by using doubling dilutions of antibody and 8 hemagglutination units (HAU) of virus, again in 96-well V-bottomed plates.

Definition of nonresponsiveness. Nonresponsiveness was defined as the failure of the postvaccination HAI titer to reach an HAI titer of 40, the titer widely accepted as the 50% protective level [8], and to have at least doubled from the prevaccination titer (some donors had an HAI titer >40 HAU before vaccination). Nonresponders failed to respond to any component of the trivalent influenza vaccine.

Molecular HLA typing. All samples were HLA class I and class II, typed by the polymerase chain reaction sequence-specific primer (PCR-SSP) method, and were genotyped for 3 common polymorphisms in both tumor necrosis factor (TNF) (+488, −238, and −308) and lymphotoxin (+720, +365, and +249). DNA samples were extracted from blood clots by use of a modified salting-out method [9]. The PCR-SSP method was done as described elsewhere; all primer sequences and concentrations have been published [9, 10].

Statistical analysis. The Fisher's exact test and odds ratio (OR) analyses were carried out with Graphpad Prism software (version 2.0; Intuitive Software for Science). $P < .05$ with a 95% confidence interval (CI) was considered significant.

Results

Frequency of nonresponsiveness to influenza vaccine. We investigated the responses of 73 at-risk donors to trivalent influenza vaccine. For the purposes of this study, a response was defined as a postvaccination HAI titer >40 (the level widely believed to indicate protection from infection) [8] and at least double the prevaccination titer. According to these criteria, 41 (56%) of 73 donors responded to ⩾1 of the 3 hemagglutinin molecules in the vaccine (11 responded to 3/3, 10 to 2/3, and 20 to 1/3 hemagglutinins); 32 (44%) of the 73 donors failed to mount an HAI antibody response to any of the hemagglutinin molecules (nonresponders).

An analysis of the effects of donor age and at-risk conditions on HAI responsiveness showed no statistically significant difference in the response rate of subjects by age (<65, 65–75, and >75 years) or between donors with different individual at-risk conditions. Donors who had not been vaccinated against influenza in the previous season had a similar response rate (60%) to those who had been vaccinated the previous season (56%).

HLA class II associations with nonresponsiveness to influenza vaccine. Responder and nonresponder HLA class II profiles were analyzed, and the data are summarized in table 1. A striking finding was a significant increase in the frequency of the common HLA-DRB1*07 allele among the nonresponders (13 [41%] of 32) compared with the responders (6 [14.6%] of 41; $P = .016$,

Table 1. HLA alleles and hemagglutination-inhibition response to influenza subunit vaccination in unrelated at-risk donors.

HLA allele	Nonresponders ($n = 32$)[a]	Responders ($n = 41$)[a]	P[b]	OR	95% CI
DRB1*0101	5 (0.16)	8 (0.20)	NS[b]		
DRB1*0103	2 (0.06)	3 (0.07)	NS		
DRB1*03	8 (0.25)	9 (0.22)	NS		
DRB1*04	12 (0.38)	15 (0.37)	NS		
DRB1*07	13 (0.41)	6 (0.15)	.016	4.0	1.3–12.2
DRB1*08	1 (0.03)	0	NS		
DRB1*09	1 (0.03)	0	NS		
DRB1*10	0 (0.00)	1 (0.02)	NS		
DRB1*11	4 (0.13)	9 (0.22)	NS		
DRB1*12	2 (0.06)	2 (0.05)	NS		
DRB1*13	3 (0.09)	15 (0.37)	.013	0.18	0.05–0.69
DRB1*14	1 (0.03)	0	NS		
DRB1*15	8 (0.25)	8 (0.20)	NS		
DRB3*0X	19 (0.59)	34 (0.83)	.035	0.36	0.10–0.88
DRB4*0101-5	19 (0.59)	16 (0.39)	NS		
DRB4*0103102n	3 (0.09)	0	NS		
DRB5*01/02/03	8 (0.25)	8 (0.20)	NS		
DQB1*0201/2	15 (0.47)	15 (0.37)	NS		
DQB1*0301/4	13 (0.41)	16 (0.39)	NS		
DQB1*0302/7/8	5 (0.16)	11 (0.27)	NS		
DQB1*0303	6 (0.19)	0	.005	20.4	1.1–376.8
DQB1*0401/2	1 (0.03)	0	NS		
DQB1*0501/4	7 (0.22)	12 (0.29)	NS		
DQB1*0602/10/11	9 (0.28)	9 (0.22)	NS		
DQB1*0603-9/14	2 (0.06)	15 (0.37)	.0023	0.0023	0.02–0.55

NOTE. CI, confidence interval; NS, not significant; OR, odds ratio.

[a] Data are no. (%). Total of HLA-DRB1, DRB3-5, and DQB1 alleles shown are less than twice the no. of donors in each group because of homozygosity and the presence of DRB1 alleles that are not associated with specific DRB3-5 genes.

[b] Two-tailed Fisher's exact test.

Fisher's exact test; OR, 3.99; 95% CI, 1.31–12.20). In the United Kingdom, the frequency of HLA-DRB1*07–positive donors is 26.5% (K.I.W. and M.B., unpublished data). In addition, an increased frequency of HLA-DQB1*0303 was observed (6 [18.7%] of 32 nonresponders vs. 0 of 41 responders; $P = .005$; OR, 20.4; 95% CI, 1.1–377), which is part of an HLA-DRB1*07–containing haplotype. There was no significant difference between the nonresponders and responders in the frequency of HLA-DRB4*01 and DQB1*02, which are also in linkage disequilibrium with HLA-DRB1*07. Further analysis of class I (HLA-A, -B, and -Cw) and class III (TNF and lymphotoxin) genes failed to reveal any additional associations (data not shown).

There was also a decrease in HLA-DQB1*0603-9/14–positive donors among the nonresponders (2 [6.2%] of 32 vs. 15 [36.5%] of 41; $P = .0023$; OR, 0.12; 95% CI, 0.02–0.55). Although the 2 HLA-DQB1*0603-9/14 subtypes (DQB1*0603/14 and DQB1*0604-9/12) were equally represented among the responders, none of the nonresponders was HLA-DQB1*0604-9/12 positive. There was also a decrease among the nonresponders in the linked alleles HLA-DRB1*13 (3 [9.4%] of 32 nonresponders vs. 15 [36.5%] of 41 responders; $P = .013$; OR, 0.179; 95% CI, 0.047–0.69) and HLA-DRB3 (19 [59.3%] of 32 nonresponders vs. 34 [82.9%] of 41 responders; $P = .035$; OR, 0.30; 95% CI, 0.10–0.88). In the United Kingdom, the frequency of HLA-DRB1*13–positive donors is 18.4% (K.I.W. and M.B., unpublished data). An investigation of DRB1*13 subtypes (DRB1*1301/16/27/28 and DRB1*1302/34) revealed no further associations. Finally, an analysis of donor HLA-DPB1 types also failed to reveal any associations with nonresponsiveness to influenza vaccine (data not shown).

Discussion

Influenza vaccination reduces morbidity and mortality in at-risk groups, but it is not universally protective [4]. The commonly used subunit vaccines contain hemagglutinin with variable quantities of neuraminidase. The protection provided by these vaccines is thought to act principally through HAI antibodies [8], since they are poor at eliciting cytotoxic T lymphocyte responses [5]. Production of IgG antibodies by B cells requires CD4 T cell help [6] and, because CD4 T cells recognize antigen in association with HLA class II molecules [7], polymorphism in these genes has the potential to modulate immune responses to subunit vaccines.

In this study, we investigated a cohort of adults who are at risk for influenza, as defined by the ACIP [3]. We found an increased frequency of HLA-DRB1*07 and a decreased frequency of HLA-DQB1*0603-9/14 and DRB1*13 in nonresponders to influenza subunit vaccine, when compared with matched responders to the same vaccine. The finding that HLA-DRB1*07 is overrepresented among persons who fail to mount a neutralizing antibody response to influenza is important because it potentially identifies a group who may not be protected by current vaccination strategies.

There may be several possible mechanisms by which HLA class II genes might modulate antibody responses to the subunit vaccines. First, the defect may be in the presentation of appropriate antigens to CD4 T cells: Persons who carry HLA-DRB1*07 may fail to recognize peptide epitopes exhibited by the subunit vaccines either because suitable epitopes are not present or are not appropriately processed. By the same mechanism, HLA-DQB1*0603-9/14 and/or DRB1*13 (which were associated with responsiveness) may be particularly efficient at the processing and/or presentation of these antigens. We previously investigated CD4 T cell recognition of influenza A/Beijing/32/92 (H3N2) hemagglutinin following both natural infection [11] and subunit vaccination [12] and found that HLA-DRB1*07 can bind synthetic peptides spanning the sequence of this hemagglutinin and that HLA-DRB1*07–restricted CD4 lymphocytes recognize and proliferate to these peptides [13].

A second explanation for the lack of an HAI antibody response is that nonresponsiveness is a more general phenomenon relating to CD4 T cell–derived help for B cells that is necessary for antibody production. The nature of such a defect is unclear, but it is compelling in that HLA-DRB1*07 has also been associated with low responses to hepatitis B vaccine, another highly purified soluble antigen [14]. Other studies have indicated that the association with nonresponsiveness to hepatitis vaccine is with the HLA-DRB1*07/DQB1*0202 haplotype [15]. Thus, it is conceivable that persons with the HLA-DRB1*07 gene may have a more general defect in antibody responses to soluble antigens. It will be important to determine whether nonresponders to influenza vaccine respond normally to other vaccines (including whole-virus preparations and live vaccines). Because a significant minority of donors with the HLA-DRB1*07 gene mount a normal antibody response to influenza vaccine, the deficit is more likely to be in a gene linked to HLA-DRB1*07 than in the DRB1*07 gene itself.

In conclusion, we found a positive association between nonresponsiveness to influenza subunit vaccine and HLA-DRB1*07 and a negative association with HLA-DRB1*13 and HLA-DQB1*0603-9/14. We believe this is the first report that polymorphisms in HLA class II molecules modulate the human antibody response to influenza vaccines. Clearly this study needs to be repeated in other populations, and, given the ongoing burden of influenza and the ever present threat of a future pandemic, it is important to further explore the phenomenon of nonresponsiveness to vaccine in order to rationally design future influenza vaccination strategies.

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